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# The New FDA Guidance for Industry "Pyrogen and Endotoxins Testing: Questions and Answers"

October, 2012

## LALUpdate volume 28, number 1

### Letter From The Editor

*On June 29, 2012 FDA released the long awaited guidance document on pyrogen and endotoxin testing, 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." It is interesting to note the broader scope of the new document. Unlike the withdrawn guidance documents, it includes pyrogen testing in the title and one of the questions addresses pyrogen testing and when it is appropriate to use this test. However, within that broader scope the question and answer (Q & A) document focuses on specific issues and does not claim to cover the breadth of endotoxin (or pyrogen) testing, as is made clear in the introduction to the document.*

*In this article most of the text of the Q & A document is reproduced in italics and comments are provided after each section. Note that in the original Q & A references are given as footnotes at the bottom of each page. In this article they have been consolidated at the end of the text. Note that a number of citations are repeated several times.*

*We would like to hear your comments on the new Q & A document. Please send them to [mdawson@acciusa.com](mailto:mdawson@acciusa.com).*

*With best wishes,*

*Michael Dawson, Ph.D., RAC*



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# Guidance for Industry Pyrogen and Endotoxins Testing: Questions and Answers

[www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM310098.pdf](http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM310098.pdf)

## I. INTRODUCTION

*This guidance provides recommendations for biological product, drug, and device firms on FDA's current thinking concerning the testing recommendations and acceptance criteria in the United States Pharmacopeia (USP) Chapter <85> Bacterial Endotoxins Test,<sup>2</sup> USP Chapter*

*<161> Transfusion and Infusion Assemblies and Similar Medical Devices,<sup>3</sup> and the Association for the Advancement of Medical Instrumentation (AAMI) ST72:2002/R2010, Bacterial Endotoxins—Test Methodologies, Routine Monitoring, and Alternatives to Batch Testing (AAMI ST72).<sup>4,5</sup> These three documents describe the fundamental principles of the gel clot, photometric, and kinetic test methods, and recommend that appropriate components and finished products be tested for the presence of pyrogens and endotoxins.*

*This guidance does not cover the entire subject of pyrogen and endotoxins testing. Instead, it addresses those issues that may be subject to misinterpretation and are not covered in compendial procedures or in currently available guidance documents. You should already have a thorough understanding of these documents when using this guidance.*

*FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word should in Agency guidances means that something is suggested or recommended, but not required.*

**Comment:** Note that the current revision of the ANSI/AAMI standard ST72 is 2011, not 2002/R2010. While the principles of the two versions of ST72 are generally consistent, the new revision contains additional information and it is recommended that the current version be used.

## II. BACKGROUND

*For more than 30 years, FDA has accepted the use of a Limulus Amoebocyte Lysate (LAL) test for endotoxins in lieu of the rabbit pyrogens test. In November 4, 1977, Federal Register notice (42 FR 57749), FDA described conditions for using LAL as a finished product test.<sup>6</sup> By 1983, FDA indicated in guidance that an LAL test could be used as a finished product test for endotoxins. These tests were described in a series of draft and final guidance documents. The last guidance document, 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, was published in 1987 (the 1987 Guidance).*

*FDA has found that the published USP and AAMI documents describing methods and calculation of pyrogen and endotoxins testing limits<sup>7</sup> provide industry with appropriate information. We also note the continued development of USP Chapters <85> and <161> and FDA guidance documents. The Agency has withdrawn the 1987 Guidance because it no longer reflects the Agency's current thinking on the topic. However, because the compendial chapters and standards do not address certain regulatory perspectives, FDA is providing supplemental information in this guidance to explain our current thinking regarding the submission and maintenance of pyrogen and endotoxins testing for FDA-regulated products.*

**Comment:** The Background section does not mention of 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.

## III. QUESTIONS AND ANSWERS

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

*The current good manufacturing practice (CGMP) regulations for finished pharmaceuticals and the medical device quality system regulations require development of controls that include scientifically sound and appropriate sampling plans.<sup>8,9</sup>*

*Sampling plan information is addressed in AAMI ST72, but not USP Chapter <85>. Firms should include a sampling plan as part of their application documentation. In the sampling plan, firms should consider the potential for contamination in raw materials, in-process materials, and the finished product. Specifically, firms should take into account aspects of the manufacturing design, including consistency of a manufacturing process, impact of in-process hold times, endotoxins removal steps, and finished product endotoxins specifications. The sampling plan should be considered dynamic; firms should begin with maximum coverage and adjust their sampling plans as they gain confidence in the prevention of endotoxins in their manufacturing processes. Firms should update their regulatory filings when adjusting sampling plans. For drugs and biological products, in-process changes to sampling plans are annual reportable changes.<sup>10</sup> For devices, a 30-day notice<sup>11</sup> may be appropriate for in-process changes to the sampling plan.<sup>12</sup>*

**Comment:** The 1987 Guideline stated "Sampling technique selected and the number of units to be tested should be based on the manufacturing procedures and the batch size. A minimum of three units, representing the beginning, middle, and end, should be tested from a lot." The new wording puts more emphasis on the justification of an appropriate sampling technique. This might result in the need for an increased level of testing until an appropriate history of testing has been documented. There is no mention in the response to Question 1 of sampling from the beginning, middle, and end of a production run, though there is in the response to Question 4.

Note that in reference 7 the ANSI/AAMI standard ST72 is mixed in with the reference for USP chapter <161>. The number of the standard is missing. In addition, as stated in the first comment on section I. INTRODUCTION, the current revision of the standard is 2011, not 2002/R2010.

### 2. When is retesting appropriate?

*When conflicting results occur within a test run, firms should consult USP Chapter <85>, Gel Clot Limits Test, Interpretation, for guidance on repeat testing. As specified in Chapter <85>, if the test failure occurred at less than the maximum valid dilution (MVD), the test should be repeated using a greater dilution not exceeding the MVD. A record of this failure should be included in the laboratory results. If a test is performed at the MVD and an out-of-specification (OOS) test result occurs that cannot be attributed to testing error, the lot should be rejected.<sup>13</sup> All testing procedures, including those for retesting within the above limits, should be specified in advance in written standard operating procedures approved by the firm's quality control unit.*

**Comment:** Previously, FDA speakers have stated at meetings that the outdated provisions for retesting in the former guidance

documents were a principle reason for their withdrawal. The FDA guidance documents were written before the Barr decision of 1993 and before the Out of Specification (OOS) Guidance issued by FDA in 2006. They were inconsistent with the more recent OOS guidance and current FDA thinking. The new document now provides consistent guidance and refers to the 2006 OOS Guidance document.

### 3. Is sample storage and handling important?

*Yes. The ability to detect endotoxins can be affected by storage and handling. Firms should establish procedures for storing and handling (which includes product mixing) samples for bacterial endotoxins analysis using laboratory data that demonstrate the stability of assayable endotoxins content. Protocols should consider the source of endotoxins used in the study, bearing in mind that purified bacterial endotoxins might react differently from native sources of endotoxins.*

**Comment:** Sample storage and handling is a point about which FDA has shown consistent concern for over 20 years (for example see 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 amoebocyte lysate (LAL) test. *J. Parenter. Sci. Technol.* 43(4):183-187). This issue should be considered, if it has not already been done. Sample storage and handling were mentioned in the LAL Update article "Laboratory Disposables and the LAL Test, Volume 22(1), p. 1. Sample stability is also mentioned in the response to question 4 below in the discussion of medical device extracts.

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

*Yes. With some exceptions (see below), finished drug product units may be pooled into a composite sample and assayed for bacterial endotoxins. The composite sample may be represented by the entire unit or partial aliquots (equal volumes) of finished product containers from one manufactured lot of aqueous-based pharmaceuticals. Pooling would generally be accepted for small-volume parenterals (those with volumes of 100 mL or less) as long as the MVD is adjusted to a proportional, lower value because of the potential for diluting a unit containing harmful levels of endotoxins with other units containing lower, less harmful, levels of endotoxins. This "adjusted MVD" is obtained by dividing the MVD computed for an individual sample by the total number of samples to be pooled. FDA suggests pooling no more than three units per composite in keeping with the concept of testing representative beginning, middle, and end finished product containers. If this reduction in MVD results in an inability to overcome product-related assay interference because of an insufficient dilution, then the samples should be tested individually.*

*Finished medical devices may also be pooled into a composite sample and assayed for bacterial endotoxins. Testing for medical devices should be conducted using rinsing/eluting and sampling techniques as described in ISO 10993-1<sup>14</sup> and ISO 1099312,<sup>15</sup> as also used for inhibition/enhancement. Sampling can be adjusted for special situations. After a suitable eluate/extract pool is obtained from a finished production lot, this pooled extract should be kept under conditions appropriate for stability until it is tested in duplicate.*

*FDA recommends that pooled samples be a composite of aseptically removed aliquots (after at least 30 seconds of vigorous mixing) from each of*

*the product containers.<sup>16</sup> In this way, the original, individual containers will be available for possible retesting in the event the pooled sample displays an OOS result.*

*Some product types should not be pooled. Two examples are drug products that have an initial low MVD (see discussion above of "adjusted MVD") and products that are manufactured as a suspension, because sample aliquot homogeneity may present significant interference issues. FDA also does not recommend pooling in-process samples from different in-process stages of the manufacturing process because it may be difficult to ensure the homogeneity of these materials.*

**Comment:** Pooling of samples and the adjustment of the MVD for drug products are issues that FDA speakers have raised at meetings for many years and it has appeared in the handouts of presentations. This is the first time that pooling of drug products have appeared in a guidance document. When testing individual vials/containers of drug product (not pooled units), there is no need to reduce the MVD (and, by implication, tighten the endotoxin limit for the product). Also, testing individual units gives more information about variability between samples; that information is lost when vials are pooled.

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

*Yes, firms may use alternative methods and/or procedures if they provide advantages in terms of accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, and in other special circumstances. Such alternative procedures and methods should be validated as described in the USP General Chapter <1225>, Validation of Compendial Procedures,<sup>17,18</sup> and should be shown to achieve equivalent or better results.<sup>19</sup> When 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.<sup>20</sup>*

*Below are two examples of alternative assays.*

#### (1) Recombinant Horseshoe Crab Factor C Assay

*If a manufacturer chooses to use a recombinant factor C-based assay, then method validation should be in accordance with the requirements of USP Chapter <85>, Bacterial Endotoxins Test, as described in the section for Photometric Quantitative Techniques, and USP Chapter <1225>, Validation of Compendial Procedures.<sup>21</sup>*

#### (2) Monocyte Activation Type Pyrogen Test

*Product-specific validation is necessary to establish whether a particular test substance or material is appropriate for evaluation of the monocyte activation method. The validation should include, but is not limited to, interference testing, accurate detection of pyrogen in individual test samples, and, for devices, ability of test system to provide direct contact to the monocytes.*

**Comment:** This response is clear and consistent regarding non-compendial methods.

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

*The transition between tests that measure the same entity (e.g., LAL cascade) can be made by comparing the two tests to verify the equivalence of the new method.<sup>22</sup> The comparison of the limit of detection and inhibition/enhancement is fundamental. The sensitivity of the new method can be evaluated on spiked product samples.<sup>23</sup> In addition to using spiked samples,*

a battery of field samples of product found to be positive may be a good source for comparing results from the methods. The method validation should also attempt to address the variability found in the normal use of the method and the manufacturing environment (e.g., source materials or seasonal changes).<sup>24</sup>

For drug, animal drug, and biological products, the transition to a new method should be submitted in a prior approval supplement (PAS). Alternatively, once a firm has established a general method for making the transition between tests, it may submit the method for review in a PAS—compatibility protocol (CP). The CP should describe, in detail, the methods used to transition between assays and the acceptance criteria used to establish the equivalence of the new method. After approval of the CP, results of implementation of the CP may be directed to be reported in a reduced reporting category (Supplement—Changes Being Effected or Annual Report or Special Report (21 CFR 314.80)). The firm should maintain the study protocol, final report, and all data at the facility for FDA review. The firm should confirm the filing process with the appropriate review division before submitting a CP. For Class III devices, the transition to a new assay requires a 30-day notice filed under 21 CFR 814.39(e). See FDA's guidance, *Modifications to Devices Subject to Premarket Approval (PMA) - The PMA Supplement Decision-Making Process*, <http://www.fda.gov/downloads/1MedicalDevices/DeviceRegulationandGuidance/UCM089360.pdf>. Manufacturing changes for Class I and II devices should be in accordance with the quality system regulation, 21 CFR part 820. Design control, production and process control requirements can be found at 21 CFR 820.30, 21 CFR 820.70, 21 CFR 820.72, and 21 CFR 820.75.

For devices, a 30-day notice may be appropriate for changes to quality control testing used on incoming components, raw materials, the in-process device, or the finished device, including performing end-product pyrogen testing on nonsterile samples prior to sterilization.<sup>25</sup> Manufactures of medical devices should demonstrate a sensitivity that is consistent with the route of administration for the device and the type of body contact. Manufacturers may use another endotoxin test after demonstrating a reproducible correlation between methods and the USP reference standard.

**Comment:** This response means 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. The response is not explicit as to 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 Q & A are those that are included in the USP BET chapter, which 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. The reagent transfer protocol available from Associates of Cape Cod, Inc., can assist with this process.

## 7. What happened to the endotoxins limit table in Appendix E of the 1987 Guidance?

The endotoxins limit table is out of date due to the increase in numbers of dosage (regimes) and drug strengths since the publication of the 1987 Guidance. The appropriate way to establish the endotoxins limit is to use the calculation methods provided in the USP or AAMI standards. Monograph limits may also not account for current product strengths or dosage regimes; these should also be checked using the calculations recommended in the standards.

If there are several components in a finished product, then the overall endotoxins limit for parenterally-administered products should not exceed the overall threshold limit specified in the USP <85> Bacterial Endotoxins Test, regardless of an individual component endotoxins limit. Intrathecally-administered products, ophthalmics, or devices (see question 11 for devices) may have endotoxins limit requirements that are not based on the calculation for parenterally administered products. FDA encourages firms to check with the appropriate office or review division about these products.

**Comment:** 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 would be prudent to verify endotoxin limits by recalculating them using information in the package insert for the product.

## 8. How can Quality by Design concepts support endotoxins limits? <sup>26</sup>

When implementing Quality by Design concepts, the strategy for endotoxins testing should be based upon product and process understanding in combination with risk management to ensure consistent final product quality. The appropriate in-process testing should be used to evaluate the production process areas at risk of endotoxins formation or incursion. Many firms already have programs for monitoring incoming ingredients and components, including the processing water, for endotoxins contamination. The finished product release specification should be considered when determining in-process limits for each phase of manufacturing tested. For purposes of evaluating the relative risk of product contamination, quantitative testing may be preferable to limit testing to facilitate product quality trending and to identify and correct excursions before they exceed the specification and cause product failure. An endotoxins limit should be justified on a case-by-case basis, and will be evaluated as a part of each relevant marketing application or supplement.

**Comment:** This item reinforces the FDA's focus on process control. It stresses the importance of endotoxin testing of raw materials, product components and in-process samples in assuring the quality of finished product.

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

For certain biological products, 21 CFR 610.13(b) requires a rabbit pyrogen test. The requirement in 21 CFR 610.13(b) may be waived if a method equivalent to the rabbit pyrogen test is demonstrated in accordance with 21 CFR 610.9.

For human and animal drugs, some USP monographs still require a rabbit pyrogen test. Even with such monographs, a firm may substitute an endotoxins test or alternative cell-based test if the firm can demonstrate equivalent pyro-

gen detection. The appropriate FDA review division will consider alternative methods, such as monocyte activation, on a case-by-case basis.

For devices and drug materials, firms should assess the risk of the presence of nonendotoxin pyrogens. If the risk assessment indicates that non-endotoxin pyrogens may be present, it may be more appropriate to use the rabbit pyrogen test.

Bacterial endotoxins assays are subject to a variety of interferences related to the physical and chemical properties of the test article. Where such interferences cannot be mitigated through sample dilution (up to the MVD) or other validated means of sample preparation, firms should use the rabbit pyrogen test.

**Comment:** This response raises the possibility of contamination of products by non-endotoxin pyrogens. While this cannot be ruled out, actual cases of this occurring 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.)

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

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 label indicates that the product may be used on juvenile and adult animals, the juvenile is considered the worst case. If the weight of the animal is required to calculate the dose, firms should use an average weight for that species. For a listing of some average animal weights, see the FDA draft guidance for industry on *Waivers of In Vivo Demonstration of Bioequivalence of Animal Drugs in Soluble Powder Oral Dosage Form Products and Type A Medicated Articles*. For animal weights not listed in that guidance, please contact FDA's Center for Veterinary Medicine.

**Comment:** The statement that "the endotoxin limit of a veterinary product should be based on the maximum product dose used on the smallest species" is surprising as it leaves open the potential for confusion if the maximum dose per unit mass is given for a larger species.

#### 11. What are the endotoxins limits for medical devices?

The Center for Devices and Radiological Health (CDRH) has adopted the USP Endotoxin Reference Standard and limits for medical device extracts expressed in EU/mL. USP Chapter <161> *Transfusion and Infusion Assemblies and Similar Medical Devices* provides the limits for medical devices within its scope. The endotoxins limit for a medical device is dependent on the intended use of the device and what the device contacts (e.g., blood, the cardiovascular system, cerebrospinal fluid, intrathecal routes of administration, permanently implanted devices, and devices implanted subcutaneously).<sup>27</sup>

For medical devices, using the extraction volume recommendations described below, the limit is 0.5 EU/mL or 20 EU/device for products that directly or indirectly contact the cardiovascular system and lymphatic system. For devices in contact with cerebrospinal fluid, the limit is 0.06 EU/mL or 2.15 EU/device. For devices that are in direct or indirect contact with the intraocular environment, a lower endotoxins limit may apply. Please contact the appropriate review division for specific recommendations.

The process of preparing an eluate/extract for testing may vary from device to device. Some medical devices can be flushed, some may have to be immersed, while others may need disassembly. Unless otherwise directed by another compendial standard, our recommended rinse volumes include

the following: (1) each of the 10 test units should be rinsed with 40 mL of non-pyrogenic water; (2) for unusually small or large devices, the surface area of the device that contacts the patient may be used as an adjustment factor in selecting the rinse or extract volume. The endotoxins limit can be adjusted accordingly. In any case, the rinse/extract procedure should not result in a greater dilution of endotoxin than recommended in USP <85>. For inhibition/enhancement testing, both the rinse/extract solution and the device eluate/extract should be tested.

Examples of medical devices with testing or interference challenges include devices that are coated with anticoagulant, contain heavy metals, or that have particulates. In these situations, treatments for interferences can include digestion, dilution, and addition of buffers, centrifugation, or filtration.

During the same surgical procedure or placement in the same surgical site, multiple units of the same device from one manufacturer should generally meet the same endotoxins limit as a single device administered during the procedure. In instances where multiple units of the same device are known or intended for use in a single procedure, manufacturers should justify any deviation from the overall endotoxins limit identified in this guidance.

When a manufacturer of medical devices plans to use LAL testing that deviates significantly from this guidance or recognized standard, a premarket notification (510(k)) under section 510(k) of the Federal Food, Drug, and Cosmetic Act (the Act) or a premarket approval application (PMA) supplement under section 515 of the Act should be submitted. Significant deviations include, but are not necessarily limited to: higher endotoxin concentration release criteria, sampling from fewer than three (3) lots for inhibition/enhancement testing, lesser sensitivity to endotoxins, and a device rinsing protocol resulting in greater dilution of endotoxins than that recommended in this guidance.

**Comment:** In addition to the limits given in USP chapter 161 of 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, CSF), the response also gives limits of 0.5 EU/mL and 0.06 EU/mL. These limits are linked to the extract volume of 40 mL recommended in the next paragraph. Provision for reduced or increased volumes is made to accommodate smaller or larger medical devices. (The 40 mL/device volume is derived from the need for sufficient volume to administer 10 EU/kg to rabbits in a pyrogen test). 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. Also, it should be noted that when an extract volume of 40 mL is used in the equation from USP chapter <161> for a device that contacts the CSF, the resulting endotoxin limit is 0.05375 EU/mL. This is more stringent than the limit of 0.06 EU/mL that is stated in the response above.

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

When the 1987 guideline document was withdrawn the explanation was lost regarding the fact that the endotoxin limit for medical devices allows for the possibility that when extracts are pooled, all of the endotoxin could come from a single device. This point is explicitly addressed in ANSI/AAMI ST72:2011.

The response to Q11 states that more stringent limits should be applied to devices for which multiple units are intended for use in a single procedure, and that the multiple units should meet the same endotoxins limit as a single device. This implies that the limit should be the limit for a single device (e.g. 20 EU) divided by the maximum number of devices likely to be used in the single procedure. This appears to indicate new thinking at FDA.

### **12. What is the FDA's expectation for regular screening of therapeutic drug products?**

*Ideally, the undiluted product should be screened as long as there is no interfering/enhancing property within the LAL test. However, in some product formulations, the ingredients interfere with the LAL test. For such formulations, the USP recommends that the product be diluted to overcome interference or enhancement properties. The calculated MVD is the dilution of a sample at which the endotoxins limit would be detected, but it should not be the regular testing dilution. When product interference is encountered during development, FDA recommends that the firm determine the lowest product dilution that would neutralize the interfering condition.*

*FDA recommends that firms begin subsequent product screening at a product dilution just above the level that neutralized the interference. For example, if the product has an MVD of 1:100, and the product displays inhibition at the 1:10, but not at the 1:20, it may be best to screen product at 1:30. If bacterial endotoxins are detected at this level, then the firm should conduct full enumeration with the product to titrate the true amount of endotoxins.*

**Comment:** This response indicates that FDA is encouraging maximum sensitivity of endotoxin tests by testing at the highest product concentration as is reasonably possible (i.e. far from the MVD).

The suggestion to test a product at a dilution of 1:30 when the first dilution that does not interfere with the test is 1:20 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.

### **13. Are control standard endotoxins still acceptable for use in running bacterial endotoxins tests?**

*Control standard endotoxins (CSEs) are endotoxin preparations other than the international or national reference standards that are traceable in their calibration to the international reference endotoxins standard. CSEs may be secondary or tertiary standards and are usually manufactured and certified by an LAL reagent manufacturer for use with a specific lot of reagent under defined assay conditions. CSEs have become an accepted source for preparation of standard curve calibrators and as assay controls, and have provided a cost saving to LAL users and helped to preserve the inventory of primary standards. FDA encourages the continued use of CSEs that are suitably calibrated to the international reference endotoxins standard.*

**Comment:** In this response FDA has provided 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 in the withdrawn 1987 and 1991 documents are not addressed.

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 new Q & A document. The USP BET chapter specifies verification of the performance of each lot of LAL reagent, but not qualification of laboratory and analysts.

Product standard curves are not included in the new Q & A document. They are mentioned in the European Pharmacopoeia, chapter 5.1.10. "Guidelines for Using the Test of Bacterial Endotoxins."

Perhaps the most notable omission is the lack of mention of archived standard curves and 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.

### **Conclusion**

The new Q & A document refers to the USP chapter <85>, Bacterial Endotoxins Test and to the ANSI/AAMI standard, ST72. It does not fundamentally change any aspect of endotoxin testing. Consequently, it is not expected to radically alter the endotoxin test. The introduction makes it clear that the document is not intended to be all inclusive and that it only addresses a limited number of issues. Amongst topics that are not included is use of archived standard curves (and controls to verify their validity). As well as providing useful information on a number of issues, the Q & A document contributes to a climate in which firms will likely be 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.

## References:

1. *This guidance has been prepared by the Division of Manufacturing and Product Quality, Office of Compliance, in the Center for Drug Evaluation and Research (CDER) in cooperation with the Center for Biologics Evaluation and Research (CBER), the Center for Veterinary Medicine (CVM), the Center for Devices and Radiological Health (CDRH), and the Office of Regulatory Affairs (ORA) at the Food and Drug Administration.*
  2. *United States Pharmacopeia (USP), 2011, Chapter <85>, Bacterial Endotoxins Test.*
  3. *USP, 2011, Chapter <161>, Transfusion and Infusion Assemblies and Similar Medical Devices.*
  4. *Association for the Advancement of Medical Instrumentation (AAMI), 2002/R2010, Bacterial Endotoxins — Test Methodologies, Routine Monitoring, and Alternative to Batch Testing.*
- Comment:** Note that the standard number, ST72 has been omitted from this reference and the current revision of the standard is ST72:2011.
5. *Veterinary drug requirements parallel the human drug requirements in safety evaluation for pyrogenicity (21 CFR 514.1(b)(5)(v), and 514.1(b)(5)(vii)(b)).*
  6. *"Licensing of Limulus Amebocyte Lysate, Use as an Alternative for Rabbit Pyrogen Test" (42 FR 57749, November 4, 1977).*
  7. *USP, 2011, Chapter <85>, Bacterial Endotoxins Test; USP, 2011, Chapter <161>, Transfusion and Infusion Assemblies and Similar Medical Devices, Association for the Advancement of Medical Instrumentation (AAMI), 2002/R2010, Bacterial Endotoxins — Test Methodologies, Routine Monitoring, and Alternatives to Batch Testing.*
  8. *See 21 CFR 211.160.*
  9. *See 21 CFR 820.70, and 21 CFR 820.250.*
  10. *See 21 CFR 314.81(b)(2)(iv).*
  11. *21 CFR 814.39(f).*
  12. *For guidance regarding when and how to submit a 30-day notice, please refer to the FDA Guidance for Industry and FDA Staff; 30-Day Notices, 135-Day Premarket Approval (PMA) Supplements, and 75-Day Humanitarian Device Exemption (HDE) Supplements for Manufacturing Method or Process Changes (issued April 13, 2011). <http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM080194.pdf>.*
  13. *For guidance regarding how to examine results that are above acceptable limits, please refer to the FDA guidance for industry on Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production. Although this guidance is not intended to address biological assays, many of the concepts in the guidance are applicable to bacterial endotoxins testing. We update guidance documents periodically. To make sure you have the most recent version of a guidance, check the FDA Drugs guidance Web page at <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>.*
  14. *ISO 10993-1:2009 Biological evaluation of medical devices Part 1: Evaluation and testing in the risk management process.*
  15. *ISO 10993-12:2007 Biological evaluation of medical devices Part 12: Sample preparation and reference materials.*
  16. *See 21 CFR 211.84(c)(3).*
  17. *USP, General Notices 6.30. Alternative and Harmonized Methods and Procedures.*
  18. *USP, 2011, Chapter <1225>, Validation of Compendial Procedures.*
  19. *USP, General Notices 6.30. Alternative and Harmonized Methods and Procedures.*
  20. *USP, 2011, Chapter <85>, Bacterial Endotoxins Test.*
  21. *USP, 2011, Chapter <1225>, Validation of Compendial Procedures.*
  22. *USP, 2011, Chapter <1225>, Validation of Compendial Procedures.*
  23. *International Conference on Harmonisation (ICH) Q2(R1) Validation of Analytical Procedures: Text and Methodology (1994); USP, 2008, Chapter <1225>, Validation of Compendial Procedures.*
  24. *Thompson, M. et al., 2002, "Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis: IUPAC Technical Report," Pure Appl. Chem., Vol. 74, No. 5, pp. 835-855.*
  25. *21 CFR 814.39(f).*
  26. *ICH Harmonised Tripartite Guideline, Q8(R2) Pharmaceutical Development, Step 4 Version (2009).*
  27. *USP, 2011, Chapter <161>, Transfusion and Infusion Assemblies and Similar Medical Devices.*



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