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Withdrawal of FDA Guideline

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Letter From The Editor

Dear LAL User:

This issue of the LAL Update addresses a milestone event in the history of endotoxin testing. After almost 24 years of service, FDA has withdrawn the venerable 1987 "Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test For Human and Animal Parenteral Drugs,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". With it has gone the 1991 "Interim Guidance for Human and Veterinary Drug Products and Biologicals: Kinetic LAL Techniques." FDA determined that the guideline was obsolete and withdrew it along with the 1991 interim guidance that had been incorporated into the online version.

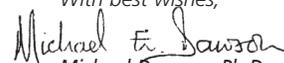
These had been important documents for those performing endotoxin testing, particularly in the late 20th century. That is before 2001, when the harmonized Bacterial Endotoxins Test (BET) chapters became official in the United States Pharmacopeia and the European and Japanese Pharmacopeia. Before the publication of the harmonized BET, the USP only addressed the gel-clot method, so the guidance documents were the primary source of regulatory information on testing by the chromogenic and turbidimetric methods.

We note that the action removes the only official document that mentioned archived or stored standard curves. Consequently, we suggest that any test using archived curves (whether in portable test systems or not) be validated as an alternative method to the procedure specified in the BET, unless already validated and/or approved.

The FDA had warned of the forthcoming demise of the guidances at a number of meetings over the last year and said that a new Question and Answer document on endotoxin testing would replace them. At the time of writing the Q and A document had not been released and there was no indication when that might be.

The article on the withdrawal looks at a number of issues that were addressed in the guidance documents and considers the impact of the removal. Where an issue is addressed in another document, that is pointed out.

If you have any questions regarding the withdrawal, or on any other matter related to endotoxin testing, please call our excellent Technical Service team or me.

With best wishes,

Michael Dawson, Ph.D.

Introduction

On July 12, 2011, FDA withdrew 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." This article addresses many of the issues affected by the withdrawal. Some of the issues are common to endotoxin testing of all types of product; others are specific to drugs and biologics or to medical devices. The article concludes with an assessment of the overall impact of the withdrawal.

In a discussion of the withdrawal on their website¹, FDA stated that "The 1987 Guideline is considered obsolete and does not reflect the Agency's current thinking on the topic." For information regarding endotoxin testing FDA refers to USP chapter <85>, Bacterial Endotoxins Test (BET)². However, there was information in the Guideline that is not included in the BET. Other sources of guidance information are referred to where appropriate.

DISCLAIMER

This article does not address every issue that was included in the 1987 FDA Guideline and the 1991 Interim Guidance documents. It includes the issues that the author considers significant; others have been omitted and it is possible that these might be important in certain situations. This article is not intended to be, and it should not be used as, a substitute for regulations or regulatory guidance. Decisions and actions should be based on the original, relevant regulations, guidance documents, standards and pharmacopeial chapters, **not** on this article.

BACKGROUND AND HISTORY

Some background information on the history of regulation of the Limulus amebocyte lysate (LAL) test for bacterial endotoxins that was in the guidance documents is still available in Annex A to the AAMI/ANSI standard, ST72:2002/(R)2010, "Bacterial endotoxins—Test methodologies, routine monitoring, and alternatives to batch testing."³ (The ANSI/AAMI ST72 standard is a recognized as a consensus standard by the FDA Center for Devices and Radiological Health [CDRH].) The withdrawal removes the description of reporting requirements when changing from the rabbit pyrogen to the BET, but changes generally are discussed in other FDA guidance documents.

LICENSED LAL REAGENT

The guidance documents stated that the LAL reagent used for endotoxin testing be licensed by the FDA Center for Biologics Evaluation and Research (CBER), but the USP BET does not include quite the same requirement. The BET specifies that the reagent be manufactured in accordance with the regulations of the compe-

tent authority, which can be interpreted to mean that LAL reagent should be licensed. The ANSI/AAMI ST72 standard does specify that all LAL reagents used in testing must be licensed and notes that in the United States LAL products are licensed by CBER.

CONTROL STANDARD ENDOTOXIN (CSE)

A significant issue regarding the withdrawal and the reference to the USP chapter is that the BET makes no mention of CSE, which had been covered in some detail in Appendix C to the 1987 Guideline. Reference to CSE was removed from the USP BET chapter when it was harmonized with the Europe and Japanese pharmacopoeia bacterial endotoxins chapters in 2001. The issue of CSEs was addressed in Pharmacopeial Forum 26(1), Jan-Feb, 2000 when the proposed text of the harmonized chapter was published. The preamble to the new text stated: "The use of in-house standards shown to be equivalent to USP Reference Standards is permitted under the requirements for alternate methods in the General Notices [section of the USP]. The control standard endotoxin (CSE) has thus been deleted because in-house standards have to be shown to be equivalent to the USP Endotoxin RS." Thus, it was clear that the USP had no intention of changing the status of CSE. However, it was also clear that use of CSE is considered an alternative to the BET procedure. The ANSI/AAMI ST72 standard does address use of CSE in endotoxin testing and states that the activity (i.e. potency) of a lot of CSE with a specific lot of LAL reagent must be determined and documented. It notes that a Certificates of Analysis giving the potency may be obtained from the reagent manufacturer.

ENDOTOXIN LIMITS, MAXIMUM VALID DILUTION (MVD) AND MINIMUM VALID CONCENTRATION (MVC)

The 1987 Guideline included a discussion of endotoxin limits, MVD and MVC. The BET does address the essentials of endotoxin limits and MVD, though with less detail and without examples. The concept of MVC (which is the concentration of the active ingredient of product at the MVD) is not addressed in the BET chapter. Further, it is not included in the ANSI/AAMI ST72 standard because MVC is generally not a parameter that applies to medical devices, which are the primary focus of the standard. However, since the MVC is a straightforward mathematical derivation from the endotoxin limit for the product and sensitivity of the test method, the withdrawal of the guidance documents does not detract from the validity or usefulness of the concept.

QUALIFICATION OF REAGENT, LABORATORY AND ANALYSTS

The qualification of reagent, laboratory and analysts was addressed in the Guideline. The USP BET chapter does include verification



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of the performance of each lot in of LAL reagent, but does not address qualification of the laboratory and analysts. However, these are general GMP requirements and Verification of Compensial Procedures is the subject of USP chapter <1226>⁴.

INTERFERENCE TESTING

Withdrawal of guidance documents for endotoxin testing removes a number of recommendations regarding interference testing (termed "inhibition and enhancement testing" in the guidance documents and the "test for interfering factors" in the BET). Some of these are common to all endotoxin tests while others are specific to either drugs and biologics or to medical devices. Each is discussed in the appropriate sections below.

Interference Testing Issues that are Common to Drugs, Biologics and Medical Devices

Number of batches upon which the test for interfering factors should be performed. The fact that this point is no longer addressed is consistent with the idea that validation is an ongoing process. However, the ANSI/AAMI ST72 standard states that studies for initial qualification/validation should be performed on at least three batches, which is consistent with what was in the 1987 guideline. Interestingly, this is under the section for the gel-clot technique, not the general section that applies to all methods.

Positive product control (PPC) concentrations for photometric techniques. Withdrawal of guidance eliminates discrepancies between the two guidance documents and the BET and thus avoids potential confusion.

Product standard curves and the specified parameters for their validity. Standard curves prepared in the material under test are not specifically mentioned in the BET. Such a strategy could be considered to fall under the general provision for sample treatments, which must be appropriately validated. Product standard curves are addressed as a means of testing samples that interfere with the test in the European Pharmacopoeia chapter, 5.1.10. "Guidelines for Using the Test for Bacterial Endotoxins."⁵

Interference Testing Issues that are Specific to Drugs and Biologics

Products with presentation in multiple concentrations of active ingredient. The guideline allowed for performing interference testing on only the highest and lowest concentrations with certain provisos. No other documents address this matter.

Determination of PPC concentration based on the pass Pass/Fail Cutoff (PFC). This point in the 1991 Interim Guidance was at odds with the PPC concentration specified in the more recent BET, so withdrawal of the guidance documents eliminates potential

confusion. (The PFC was the endotoxin limit of the dilution of products that was being tested, as opposed to that of the undiluted product.)

The number of batches of product to test and when to revalidate. The BET addresses this matter at a high level by stating the principle that (re-)validation of the test method is required when conditions that are likely to influence the test result change.

Interference Testing Issues that are Specific to Medical Devices

Grouping of devices according to common chemical composition. This principle is retained in the ANSI/AAMI ST72 standard.

Recommendations on the numbers of units to test per batch. There was a slight difference in the numbers of samples specified in the guideline and in USP chapter <161> on medical devices⁶. For lots sizes <30 the 1987 Guideline allowed testing of 2 devices (as does ANSI/AAMI ST72 which references the guideline) while USP <161> specifies that a minimum of three units be tested. The guidance documents did not address revalidation but guidance on this matter is provided in ANSI/AAMI ST72.

Performance of interference testing when batches of raw materials change. Loss of this recommendation is addressed by the more general requirement in the BET that the test for interfering factor be conducted when conditions that are likely to influence the test result change.

PERFORMING THE TEST

As with interference testing, some of the topics that were addressed in the FDA guidance documents are common to performance of the test (as opposed to its validation) for drugs, biologics and medical devices, and some which are more specific. They are addressed in the appropriate sections below.

Testing Issues that are Common to Drugs, Biologics and Medical Devices

Inclusion of a full series endotoxin standards with at least the first gel-clot test of the day. The guideline also required that a series of standards be included when test conditions change (including a change of LAL reagent lot) and for repeat tests. The BET requires only a two lambda concentration positive control with limits tests, while a full series standards is specified for the quantitative gel-clot test (i.e. with every test). The BET also requires repeating test for interfering factors (which includes a full series of standards) when any condition changes that is likely to influence the result of the test.



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Archived standard curves for photometric methods. Archived (stored) standard curves and the controls to verify their validity are not addressed in any regulatory document, guidance or standard now that the guidance documents have been withdrawn. The USP BET chapter does not include archived standard curves; it specifies that a standard series be included with every photometric test. The ANSI/AAMI ST72 standard does not mention archived standard curves either. Consequently, archived standard curves must be considered alternative methods and validated accordingly.

Repeat tests. The FDA guidance documents allowed for up to two retests, first a retest of the sample using double the original number of replicates and then testing of ten new samples, which each had to meet specifications when tested individually. The FDA guidance documents were written before the FDA Out of Specification (OOS) Guidance of 2006 and these retesting provisions were not consistent with the more recent OOS guidance. Removal of the guidance documents eliminates this inconsistency.

Testing Issues that are Specific to Drugs and Biologics

Batch sampling technique for release testing. The 1987 guideline stated that the sampling technique selected and the number of units to be tested should be based on the manufacturing procedures and the batch size and that a minimum of three units, representing the beginning, middle, and end, should be tested from a lot. No such direction is given in the BET and sampling of drugs products is not addressed in ANSI/AAMI ST72. As in other areas of testing, firms should be prepared to justify their sampling plan and the number of units tested.

Pooling of samples. The BET does not mention pooling of samples but the 1987 guideline allowed samples to be tested individually or to be pooled for testing. FDA have repeatedly stated at meetings (and in the distributed presentations) that if samples are pooled, the MVD (and thus by implication the endotoxin limit) should be reduced by a factor equal to the number of units pooled. The purpose of this adjustment of the MVD is to assure that contaminant endotoxin in one of the units is not diluted to below the endotoxin limit when pooled with uncontaminated units.

Prohibition of retesting and LAL test failures in a pyrogen test. The 2006 OOS Guidance document is a valuable resource concerning retesting of test failures.

MEDICAL DEVICES

Endotoxin Limit. An explanation of the endotoxin limits, which had become rather dated, was included in the 1987 guideline. In this respect withdrawal of the guideline eliminates a possible source of confusion. Endotoxin limits for medical devices are more clearly stated in USP chapter <161>. Unfortunately, withdrawal of

the guidance documents also removes the explanation that the limit for medical devices takes into account the following

1. All of the endotoxin in a pool of 10 devices could come from a single device. This point is implicit in USP chapter <161>, which gives an endotoxin limit of 20 EU/device (except for intrathecal devices, for which the limit is 2.15 EU/device) and gives an equation for the endotoxin limit per unit volume of extract fluid. The equation does not compensate for pooling so that when the extracts of ten devices are combined, one of the devices could be contaminated with almost 200 EU and the pooled extract solution would still meet specifications if the other devices were clean.
2. The recognition that extraction procedures are often not 100% efficient.

Extraction procedure. The withdrawal eliminates a difference between the 1987 Guideline and that in USP chapter <161>. However, the extraction procedures given in AAMI ST72 are the same as those that were in the guideline, so the discrepancy persists.

APPENDIX E

Withdrawal of the guidance documents removes Appendix E, which was a listing of doses and endotoxin limits, eliminates the potential for conflicts between limits in the Appendix, USP monographs and in product submission.

CONCLUSION

Withdrawal of the FDA guidance documents is unlikely to fundamentally change the way endotoxin testing is performed. It does eliminate some discrepancies between the guidance documents and the USP BET. Many of the issues that were addressed in the guidance documents, but not in the BET, are GMP issues that should be part of any laboratory's normal procedures. These include analyst and laboratory qualification, numbers of samples to test and repeat testing. Also, many of these issues are addressed in the ANSI/AAMI ST72 standard.

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