LAL UPDATE®

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Dear LAL User:

This issue comments on the revisions of the USP chapter on ***Transfusion and Infusion Assemblies*** *now entitled* ***Transfusion and Infusion Assemblies and Similar Medical Devices.*** *Included also is a short article on label claim sensitivity and its use in calculating endotoxin concentrations and Maximum Valid Dilution (MVD).*

We are pleased to introduce Carmen Barillas, who joins us to work in technical services. Carmen comes to us from Genica Pharmaceuticals, Worcester, MA and is a native Spanish speaker. She is a valuable addition to the team and will be particularly appreciated by our Spanish speaking customers.

We all wish you a challenging and prosperous new year. Thank you for choosing Associates of Cape Cod, Inc.

Sincerely,

Thomas J. Novitsky, Ph.D.
Editor

USP Changes
Medical Devices Chapter
Official January 1, 1995

The USP **Transfusion and Infusion Assemblies** chapter has been modified in the First Supplement to USP 23 and is now called **Transfusion and Infusion Assemblies and Similar Medical Devices**. The revised chapter changes the way endotoxin limits are expressed and eliminates most of the discrepancies between extraction procedures for different types of devices.

The limit is now stated as 20 EU/device for devices that contact the blood or lymph and 2.15 EU/device for devices that contact the cerebrospinal fluid (CSF). Formerly, the limits were expressed per unit volume of the extract fluid and were 0.5 EU/ml and 0.06 EU/ml respectively, based on a standard extract volume of 40 ml/device.

The revised chapter, like the FDA *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*, recognizes that 40 ml may not be an appropriate extraction volume for all medical devices and allows the endotoxin limit to be proportionally adjusted if the extract volume is changed. The revision takes the logical step of presenting a formula to calculate the endotoxin limit for different extraction volumes. The formula is:

$$\textit{endotoxin limit} = \frac{K \times N}{V}$$

where K is the endotoxin limit per device (for example, 20 EU/device for devices that do not contact CSF); N is the number of devices to be tested; and V is the total volume of extract or rinse (i.e. extract volume per device x number of extracts pooled).

The revision to the chapter has not changed the effective endotoxin limit for most medical devices. For example, if the "standard" 40 ml per device is used and the extracts from 10 devices are pooled, then $N = 10$, $V = 400$ ml, and the endotoxin limit is 0.5 EU/ml, the former limit.

However, for devices that con-

tact the cerebrospinal fluid, the limit has changed slightly. When the limit of 2.15 EU/device is used in the equation for 10 devices, each extracted in 40 ml, the endotoxin limit is 0.05375 EU/ml. This is less than the former limit of 0.06 EU/ml and necessitates use of a more sensitive LAL reagent than was previously required. Alternatively, the extraction volume can be reduced to 35 ml/device to retain the limit at 0.06 EU/ml.

The calculated endotoxin limit is not influenced by the number of extracts pooled because N appears on the top line of the formula and is also incorporated into V on the bottom line (V = extract volume per device x N). Consequently, the formula does not account for unequal distribution of endotoxin contamination between the devices. For example, if 10 extracts of 40 ml are pooled to give 400 ml, the total amount of endotoxin in the extract must reach 200 EU before an LAL test failure is recorded at 0.5 EU/ml. It is quite possible that all of this endotoxin could have come from a single device.

A detection limit of 200 EU/device is not a cause for concern. It has been the *de facto* limit for pools of extracts from ten devices since the BET became an official test for medical devices in the USP some ten years ago. This discrepancy, 20 EU/device for a single device vs. a worst case of 200 EU/device when 10 extracts are pooled, has been largely ignored. The FDA guideline recognizes the potential for unequal distribution of endotoxin.

Even 200 EU/device is well below the limit of 5 EU/kg x 70 kg = 350 EU per person that is the basis for endotoxin limits for drug products. The limit of 5 EU/kg is used to calculate limits per unit of product for non-intrathecal parenteral drugs and 70 kg is the average human adult weight. The FDA Guideline supports the more stringent limit for medical devices by noting that extraction procedures are rarely 100% efficient.

It is interesting that there is a move to reduce the endotoxin limits of drugs when units from a batch are pooled for testing. This is so that contaminated product in one container is not diluted to below the limit by clean material in other containers. It is likely that this will be formalized when an updated FDA guideline is published, perhaps in 1995. This logical approach contrasts with the situation for medical devices, for which endotoxin limits become, if anything, less stringent when extracts are pooled.

It is certainly not suggested that the limit for devices should be tightened when device extracts are pooled; rather to point out that pooling raises the worst case limit above 20 EU/device. Given the limit of 350 EU/person for drugs and biologicals, a good case can be made for raising the limit for devices to 200 EU/unit, and then correcting for the effects of pooling extracts. A counter argument is that limits should be tighter when lower numbers of extracts are pooled because of the reduced sample size and the "spotty" nature of contamination on at least some medical devices.

Regardless of the endotoxin limits, which are largely unchanged from the old Transfusion and Infusions Assemblies chapter, the revision represents a substantial improvement over its predecessor. The chapter has been simplified and the equation clarifies the determination of limits. Also, it is slightly shorter than the chapter it replaces, a rarity in revisions and clarifications!

Calendar

PDA Spring Meeting
Hyatt Regency
San Francisco, CA
March 13-16, 1995
Table # 82

ISPE Vendor Show
Howard Johnson's Hotel
Cambridge, MA
March 14, 1995

Center for Professional Advancement Course
"LAL Testing: Drugs, Medical Devices, and Biotechnology - Endotoxin Detection in QA/QC and Product Development"
San Francisco, CA
April 24-26,1995
Course Director-
Michael E. Dawson, Ph.D.

ASM Meeting
Washington, DC
May 21-25, 1995

LAL Label Claim Sensitivity and Its Use in Calculations

The USP **Bacterial Endotoxins Test** chapter, the European Pharmacopoeia **Bacterial Endotoxins** chapter and the FDA guideline require that the confirmed label claim be used to calculate endotoxin concentrations in gel-clot assays. It is worth reviewing exactly what label claim is, how it is determined and, most importantly, how it should be applied in calculations.

The sensitivity indicated on the label of a vial of gel-clot lysate is the least amount of FDA reference standard endotoxin (RSE) required to cause the reagent to clot under standard conditions. The labelled sensitivity is often represented by the Greek letter (lambda) λ. At Associates of Cape Cod, Inc., sensitivity is determined by testing a series of twofold dilutions of endotoxin starting from a concentration of 1 EU/ml. The concentrations in the dilution series are 1.0, 0.5, 0.25, 0.125, 0.0625 0.03125, 0.015625, 0.0078125 EU/ml. The labelled sensitivity is the geometric mean endpoint obtained in a series of endotoxin dilutions. The endpoint is the last tube in that series to clot.

Given that the accepted error of the gel-clot test is plus or minus one twofold dilution (because the test cannot resolve between the twofold dilutions), it is clearly meaningless to express all the decimals in the labelled sensitivity. By convention, sensitivities are expressed as 0.5, 0.25, 0.125, 0.06 and 0.03 EU/ml.

However, there are a number of advantages to using all the decimal places in calculations. These include:

1) Mathematically, it is more correct to use all decimal places and then round the final result as necessary, despite the fact that the number used in the calculation is not identical to that on the vial.

2) Maximum valid dilutions (MVDs) and assay results calculated using all decimal places are more frequently whole numbers than those obtained if the actual labelled sensitivity is used. For example:

A. MVD

a) calculation of the MVD using the labelled sensitivity of 0.06 EU/ml:

$$MVD = \frac{35\ EU/ml}{0.06\ EU/ml} = 583.333$$

b) calculation of the MVD using a sensitivity of 0.0625 EU/ml:

$$MVD = \frac{35\ EU/ml}{0.0625\ EU/ml} = 560$$

In the latter case the MVD is lower and more conservative because it allows for slightly less dilution of product, increasing the chance of detecting endotoxin.

B. Calculation of assay results

If a series of dilutions of sample are tested and the endpoint dilution of all replicates is at 1:64 and if the labelled Pyrotell® sensitivity is 0.03 EU/ml:

a. calculation of the endotoxin concentration using the labelled sensitivity of 0.03 EU/ml:

$$64 \times 0.03 = 1.92\ EU/ml$$

b. calculation of the endotoxin concentration using a sensitivity of 0.03125 EU/ml:

$$64 \times 0.03125 = 2.0\ EU/ml$$

3) If all decimal places are used to calculate assay results, there is no danger of releasing product which should fail the test. For example, in **B** above, if the endotoxin limit was 2 EU/ml and the full sensitivity of 0.03125 EU/ml is used, it cannot be claimed that the result is 1.92 EU/ml and that the product passes. Endotoxin concentrations calculated using all decimal places are always slightly greater than when the actual labelled sensitivity is used instead of rounded values, which is more conservative in terms of release of product.

4) Results obtained for a given sample using both less sensitive and more sensitive LAL lots are more likely to agree, or at least fall within the accepted twofold error of the test, if all decimal places are used. For example, a sample that gives an endpoint at a twofold dilution with a reagent sensitivity of 0.125 EU/ml and at a fourfold dilution with a 0.06 EU/ml reagent should be reported to contain 0.25 EU/ml in both cases.

In conclusion, we recommend that all the decimal places be used in calculations that involve label claims of 0.06 and 0.03 EU/ml.

Associates of Cape Cod, Inc.
LAL In-House Workshop

Associates of Cape Cod, Inc. sponsors two-day, in-house LAL training courses and workshops. The first day is primarily lecture. Topics covered include: the structure, activity and control of endotoxins, the biochemistry of the LAL test, LAL methods and applications, and the regulatory issues that cover setting up an LAL laboratory and performing end-product release testing.

The second day is an opportunity to work in the laboratory under supervision. There will be demonstrations of the gel-clot, turbidimetric and chromogenic methods. There will also be time to gain hands on experience in one's methods of choice. If arrangements are made in advance, individuals may bring a nonhazardous sample to develop test protocols. Users may wish to ask questions pertaining to their own SOP's or products. Time can be arranged to ask questions in private as well as in the classroom.

Dates for the next three workshops are February 21-22. March 21-22 and April 18-19, 1995.

Contact Robin McFarlin (800-848-3248 x 206) for more details.

Associates of Cape Cod, Inc.

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