



# LALTIMES



Vol. 5, No. 1, February 1998

## EDITOR'S COMMENTS

*Progressive parenteral facilities have developed a keen awareness of the need to fully utilize LAL methods for process control as well as assessment of end-product quality. Regulators perceive the scope of LAL testing to include monitoring of water systems, validation of production processes, testing of bulk pharmaceutical chemicals and other additives, and trend analysis.*

*This newsletter is the first of a multi-part series that will describe how endotoxin testing impacts on microbiological quality assessment throughout the production process. This series will focus on testing of raw materials, bulk pharmaceuticals and in-process samples because current regulations are designed for finished goods, and there is a paucity of direction for other categories of LAL testing. Your feedback to this series is most welcome. Please direct your comments to the editor via e-mail: [JFC@endo.criver.com](mailto:JFC@endo.criver.com) or by fax:803-795-7221.*

*Dr. James F. Cooper, Global Scientific Director*

**LAL INTERFERENCE SCREENING OF IN-PROCESS MATERIALS AND FINISHED PRODUCTS - J.F. Cooper**

The first step in creating a LAL-test method for a pharmaceutical entity is identification of a compatible concentration for routine testing. This discussion presents a strategy for compatibility testing and choice of

relevant test concentrations, defines terms and suggests a means for reporting the sensitivity of LAL analyses consistently. Attention is focused on in-process materials because there is a paucity of information for this type of LAL testing. These ideas apply equally to gel-clot and kinetic methods unless otherwise specified.

## VALIDATION ISSUES

**LAL compatibility** is a test condition where an endotoxin standard is detected with the same efficiency in a test sample as in LRW (LAL Reagent Water).

**Inhibition** is a test condition where less endotoxin standard is recovered than is expected, and conversely, **enhancement** is recovery of more endotoxin than expected.

The LAL gel-clot method is somewhat limited in that inhibition is the only problem observed because enhancement is rarely seen except during the validation process. Stringent controls in kinetic methods reveal both inhibited and enhanced recovery of the endotoxin control. However, enhancement in kinetic systems is usually an artifact of the standard curve caused by inadequately performing LAL or endotoxin standard.

A **positive product control (PPC)** is a known amount of endotoxin mixed with a test material to confirm the absence of interference and to render a given LAL test valid.

**Endotoxin Standards:** The BET (Bacterial Endotoxins Test) chapters in the major pharmacopoeias require a **standard dilution series**, a two-fold dilution of CSE or RSE from  $2\lambda$  to  $\frac{1}{4}\lambda$ , to verify LAL sensitivity.

Superficially, one might suspect that the series exists to show that the LAL is active. Closer scrutiny reveals that the series verifies that the analyst has conducted tests within the conditions specified by the LAL vendor and has prepared the PPCs properly.

**Validation** of LAL compatibility is a process that confirms the ability to detect endotoxin in a specific parenteral product by LAL methods without measurable interference. This process involves proving that endotoxin standards are detected equally in a test specimen as in LRW.

Part of the validation process is calculation of product specific data, such as the **endotoxin limit (EL)**, **maximum valid dilution (MVD)**, and **minimum valid concentration (MVC)**. The EL for most final products is found in pharmacopeial monographs and Appendix E (1994) of the FDA's LAL-test guideline, or is calculated from the K/M formula.

## CHOICE OF TEST CONCENTRATION

**Value of the MVC:** The minimum valid concentration is only found in formulae contained in the FDA guide. The MVC is not applicable to infusion solutions or drugs where the endotoxin limit is given in EU/mL. Rather, it applies to products where the EL relates to a quantity, such as EU/mg. The MVC is defined as the concentration of a pharmaceutical entity when it is diluted to the MVD, a value without units. The merit in using the MVC rather than MVD for sample preparation is that the MVC has units, so that samples can be prepared expeditiously for testing, regardless of the form of the original sample. For example, an analyst may need to test a specific drug product that is presented in various forms, such as a raw material, pre-fill solution or lyophilized end products in varying fill quantities. Using the MVC or a multiple thereof as a guide, the analyst can dilute directly to the validated, compatible test concentration without a redundant calculation of the MVD. Another reason for using concentration rather than dilution is that test results can be reported in the same units of the endotoxin limit.

For example, the endotoxin limit for a cephalosporin is 0.2 EU/mg. If the validated method calls for test concentration rather than a dilution, the results are reported directly in the same units, EU/mg.

**Test Sensitivity:** It is critical to clarify the sensitivity of an assay in test protocols. For LAL testing of water, infusion solutions or device extracts, only the reagent sensitivity and sample dilution are needed to calculate test sensitivity. For therapeutic drugs, the test concentration and lambda are needed to report test sensitivity. To specify the test conditions and report the results in the same units as the endotoxin limit, the expression **Product Specific Sensitivity (PSS)** is introduced, and is calculated as follows:

**PSS = Lambda ( $\lambda$  in EU/mL)**

**Product concentration (mg/mL)**

Note that sensitivity of a specific test may be increased by either selecting a reagent with greater sensitivity (lower  $\lambda$ ) or by increasing the test concentration. The upper limit of test concentration (potency) is restricted to the validated, compatible test concentration. An increased concentration may be the most practical way to increase the PSS.

**In-Process Controls:** Endotoxin limits for In-process controls of a parenteral process are selected after careful

consideration of process capability and historical data. Let us assume, for example, that a decision is made to assign endotoxin specifications for a production process to include the bulk pharmaceutical, a critical in-process point and a pre-fill solution. Before assigning in-process specifications, historical data from a minimum of three production lots should be collected before assigning endotoxin limits for critical control points or pre-fill solutions.

If a bulk pharmaceutical historically is contaminated with endotoxin, but there is an endotoxin depletion step in the production process, (recrystallization, ultrafiltration, etc.) it is justifiable to assign a rather permissive endotoxin limit to the bulk pharmaceutical. If there is no endotoxin depletion step, a more restricted limit is prudent.

Critical points suitable for in-process control include steps that may alter endotoxin content, such as depletion of endotoxin or exposure to water. Limits for these points must be no more restrictive than achievable by the process, based on historical data.

## LAL COMPATIBILITY PROFILE

Selection of test levels vary in practice because earlier versions of the USP's BET urged testing at the least, non-inhibitory dilution, whereas the BET of the EP suggested the MVD. Both chapters now permit testing at any valid, reasonable concentration. Selection of test potency is dependent on a LAL compatibility profile. Let's consider the following data from an interference screen for an antibiotic where a lambda of 0.05 EU/mL gives a MVC of 0.03 mg/mL. Two-fold dilutions of the drug were tested by three methods. The gel-clot and kinetic turbidimetric assay (KTA) were made with Endosafe® KTA, licensed for both methods. The kinetic chromogenic assay (KCA) was made with Endochrome K. Table I shows that recovery of the PPC increases modestly as drug concentration decreases over a 64-fold dilution range.

The goal here is choice of a concentration for validation that consistently recovers 100% of the PPC. The profile reveals that a concentration of 0.1 mg/ml is compatible with all systems, and is a good choice for validation. The standard curve of 5 to 0.05 EU/mL, selected for the preliminary screen, is quite suitable for validation and routine testing, also. For this scenario, the PSS is 0.5 EU/mg and compares favorably to the published endotoxin limit of 1.7 EU/mg.

### **Table 1: LAL Compatibility Profile for an Aminoglycoside Antibiotic**

Conc. (mg/ mL)	Gel-clot PPC	Recovery KTA (%)	Recovery KCA (%)
4	-	7	2
2	-	27	6
1	-	55	29
0.5	+	79	57
0.25	+	97	81
0.125	+	103	99

The profile yields surprising data. *First, the gel-clot method appears more compatible than the kinetic methods only because the  $2\lambda$  PPC partly masks inhibition.* The same reagent was used for gel and KTA, thereby eliminating method as a source of variation. *Secondly, an 8-fold dilution was needed to increase recovery from 50 to 100%.*

In summary, compatibility profiles related to the MVC provide a rational basis for identifying ideal concentrations for validation and routine testing of pharmaceutical samples. The PSS serves as a uniform, efficient way to report test results.

### **Fisheries Update**

On October 23 a Policy Board of the Atlantic States Marine Fisheries Commission (ASMFC) voted to address American eel and horseshoe crab management plans separately. This action was taken to assure that the horseshoe crab resource would receive adequate and timely attention. The goal of the ASMFC action is to develop an interstate management plan that will reverse declines in horseshoe crab populations in Delaware Bay and nearby waters.

## Liquid Endotoxin Standards

*Foster T. Jordan, General Manager, Charles River Endosafe*

Since the most demanding part of routine LAL testing is maintenance of endotoxin standard potency, a ready-to-use form of CSE is tantalizing in appeal. However, there are complicating regulatory constraints to this approach.

A liquid endotoxin standard has been marketed that is labeled in EU/mL rather than nanogram per unit volume or vial, and the potency is not specific to any lot of LAL reagent. Such labeling is in conflict with the Bacterial Endotoxins Test (BET) of the USP and the FDA's LAL-test Guideline. Both documents require the analyst to use either RSE or a CSE calibrated to the RSE in EU/ng. The RSE/CSE standardization, as described in Appendix A of the Guideline, is specific for each lot of LAL and CSE. An FDA field inspector would expect to see the standardization data for liquid endotoxins during a routine compliance audit because responsibility would default to the user in the absence of a vendor Certificate of Analysis. As currently presented, ready-to-use liquid standards are not suitable for a GMP facility.

At Charles River Endosafe, we endeavor to make endotoxin standards that are stable, readily dispersible, easy to use and standardized in strict compliance with the BET. In addition to high potency CSE, we make low-potency CSE to minimize time-consuming dilution and vortex mixing. Similar to the RSE formula, we include PEG in our CSE preparation to aid dispersion and a filler to promote proper lyophilization.

**CALENDAR**

**PDA LAL Technology Workshop, March 23-24, 1998**, Baltimore, MD - The workshop features a condensed one day course on LAL test fundamentals and a one day wet lab on validation techniques with an introduction to kinetic LAL methods. This course is structured for entry level and intermediate level LAL analysts who wish to broaden their knowledge.

**LAL Managers Seminar April 29 - May 1, 1998** - Florham Park, NJ. This seminar is a great opportunity for LAL decision makers to broaden their perspective on LAL practice and regulatory issues. The unique format of the meeting incorporates a scenario featuring a start-up drug company which faces complex LAL testing issues. LAL experts present problems that are addressed in break-out sessions and are reviewed by all participants. For further information contact Karen McCullough by phone, (908) 5334-8897, fax (908) 534-1317, e-mail at KarenZM@aol.com, or by mail at P. O. Box 635, Martinsville, NJ 08836.

**CREL LAL Workshop, May 20-22, 1998**, Stoke Rochford Hall, Nr Grantham, Lincolnshire: Charles River Endosafe Limited is pleased to announce a spring LAL Workshop that focuses on the expanded applications for LAL testing. For more information, contact Sara Marsh: Phone (44)1843 822331; Fax (44) 1843- 822989.

**Annual Charleston LAL Workshops, July 22-30, 1998**, Charleston, SC - The Gel-clot workshop will be held July 22-25, and the Kinetic workshop will be held July 27-30. Workshop announcements will be distributed in late February for this 20th anniversary workshop. For further information contact Frances Cooper (Phone 803-795-7316; Fax: 803-795-7221).

**Fifth Conference of the International Endotoxin Society, September 12-15, 1998**, Santa Fe, NM - This conference will include invited speakers and those chosen from submitted abstracts. The deadline for submission of abstracts for posters and presentations is March 31, 1998. For further information visit the Society web site at: <http://www.kumn.edu/IES/cshome.htm>, or contact Shirley Kolkey at c-c.m@worldnet.att.net, or by fax at 619-299-6675.