



# LALTIMES

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## **EDITOR'S COMMENTS**

*It has become traditional in LAL technology to conduct methods development on a new product by calculating the MVD, and then validating that dilution as though there were no other options. With the availability of interference-resistant LAL reagents and a choice of methods, a manager has the option to predetermine the desired margin of safety for a finished product and secure alert limits for raw materials. In this third of a series of topics on endotoxin testing in the production process, a strategy for preplanning endotoxin specifications is presented. In the course of explaining this strategy, an approach is proposed for setting limits for combination drugs.*

*A discussion of rounding of analytical results that appeared in the MAY PDA Letter prompted me to address this issue from the viewpoint of LAL users. The rounding of label-claim for lower sensitivity gel-clot reagents can often lead to underestimation of end-point determinations and BET parameters. Revision of SOPs is proposed as the most efficient means to manage this problem.*

**Dr. James F. Cooper**

## **BET CALCULATIONS FOR MULTIPLE COMPONENT PARENTERALS**

**Dr. James F. Cooper**

The calculations for endotoxin limit (EL), maximum valid dilution (MVD) and minimum valid concentration (MVC) for a drug entity are relatively straightforward when using an EL from a pharmacopoeia or Appendix E of the FDA's LAL-test guideline. However, calculation of BET parameters for raw materials, multi-ingredient drug products or multi-component dosage forms are not addressed directly in official documents. This discussion suggests ways to calculate limits for unusual dosage forms and plan for margins of safety.

A frequently asked question when LAL testing is being considered for a new product is, "What is the MVD?" A more direct approach to methods development is to select a predetermined safety factor for the LAL test. The safety factor is calculated by dividing the endotoxin limit by the

sensitivity of a specific validated test method. Refer to our FEB-98 Newsletter (*LAL Times*, **5: 1-4, No. 1, '98**) for a discussion of reporting of results and the product specific sensitivity, PSS, where:

$$\text{PSS} = \frac{\text{Lambda } (\lambda \text{ in EU/mL})}{\text{Test concentration (mg/mL)}}$$

### Safety Factors for LAL Tests

When interference isn't a limitation, It is commonplace to test finished products with a safety factor, that is, to select an alert limit that is 2 to 10 times more stringent than the endotoxin limit. A simple method is proposed here to calculate the test concentration or dilution needed to attain a larger safety margin. The test concentration (TC) or test dilution (TD) needed for greater test sensitivity, defined as the alert limit (AL), is found by rearranging the PSS formula, shown below. The choice of formula depends on whether the endotoxin limit is by quantity (EU/mg) or by volume (EU/mL):

$$\text{By weight or unit} \quad \text{TC} = \frac{\lambda}{\text{AL}} \quad \text{or by dilution:} \quad \text{TD} = \frac{\text{AL}}{\lambda}$$

### Single Ingredient Parenterals

To apply this approach to a single-ingredient drug, let us consider an antibiotic which is available in 25 mg/mL with an EL of 0.2 EU/mg. To increase sensitivity 4-fold is to test at one-fourth the EL; that is, the alert limit becomes 0.05 EU/mg. If a reagent lambda of 0.125 EU/mL were used, we can determine the test concentration needed to test at the alert limit (EL/4) by using the weight-related formula, where, the TC is found by dividing lambda by the alert limit:

$$\text{TC} = \frac{\lambda}{\text{AL}} = \frac{0.125 \text{ EU/mL}}{0.05 \text{ EU/mg}} = 2.5 \text{ mg/mL}$$

Thus, in this example, it becomes clear that a 1:10 dilution of the product (from 25 to 2.5 mg/mL) allows one to test at the alert limit when lambda equals 0.125 EU/mL.

## Multiple Ingredient Parenterals

Calculations are more challenging for a multi-ingredient drug because of the need to consider multiple endotoxin limits. There are examples of limits for multi-ingredient drugs in Appendix E. Although amino acid injections may have up to 15 different amino acids per dosage form, a uniform EL of 0.2 EU/mg is assigned for all amino acid parenterals. Also, the multi-component ACPD anticoagulant solution found in blood bags is assigned an EL of 5.56 EU/mL based on a total volume of 63 mL. Large volume parenterals (LVPs) that contain mixtures of sugars and electrolytes are assigned the general LVP limit of 0.5 EU/mL. This pattern demonstrates that it is easier to base the endotoxin limit on the total **dose volume** rather than each ingredient in a combination drug. For example, let us consider a common injectable that contains 3 types of vitamin B, as given in the formula below, where the EL is very different for each vitamin.

### Ingredient mg/mL EL (EU/mg)

Thiamin 20 3.5

Pyridoxine 200 0.4

Cyanocobalamin 0.2 400

Let us assume the labeling describes the total dose as the contents of a 5-mL ampule. Rather than calculate the MVC and MVD for each component to see which one is more stringent, it is easier to base the endotoxin limit on the 5-ml dose, such as 350 EU/5 mL, or 70 EU/mL. (It is often easier to calculate the EL from 350 EU/adult dose, rather than 5 EU/dose/kg.) To test with a safety factor that is double or five times more sensitive than the EL is to test at 35 or 14 EU/mL, respectively. The dilution required to test at an alert limit 5 times more sensitive (70 EU/mL/5 or 14 EU/mL) using a reagent 0.125 EU/mL is found by solving for TD with the volume-related formula, where,

$$TD = \frac{AL}{\lambda} = \frac{14 \text{ EU/mL}}{0.125 \text{ EU/mL}} = 112$$

For convenience, one would test a 1:100 dilution for this alert limit. Interference would not be an issue for this dilution of the multi-vitamin preparation.

A strategy for selecting an endotoxin limit for each raw material requires more thought. In the case of the above multi-vitamin ampule, the most stringent EL is the published one divided by 3 to account for each component potentially carrying the limit. This is not realistic because raw materials that are USP-grade or better rarely contain excessive endotoxin. Also, additional processing of the combination drug, such as steam sterilization, further reduces inherent endotoxin contamination. In the absence of interference, a realistic approach is to keep the published EL as the raw material specification, but set an action limit at one-

half the EL and an alert limit that is one-tenth the EL. The test concentration needed to achieve the raw-material alert limit for pyridoxine (EL/10) is determined using the weight-based sensitivity, as follows:

$$TC = \frac{\lambda}{AL} = \frac{0.125 \text{ EU/mL}}{0.04 \text{ EU/mg}} = 3.1 \text{ mg/mL}$$

This calculation demonstrates how to easily increase test sensitivity (PSS) by increasing the test concentration. To repeat the word of caution in the FEB-98 Newsletter, no alert limits should be set without collecting historical data to determine if there is incompatibility or low-level endotoxin in existing supplies that would minimize this strategy. There is no merit to setting overly stringent specifications that might limit source of supply or increase raw-material cost. Greater safety factors may not be applicable to natural or recombinant products with low-level, inherent endotoxin.

If there is no published limit for a drug entity, simply use the same EL for the bulk as the finished product, and use the above strategy for setting action and alert limits. For excipients, calculate an EL based on their proportion in the final formula and follow the same strategy for raw material specifications. These strategies assume that most active drugs and excipients are relatively free of endotoxin.

### Multiple Component Parenterals

This type of product usually has the active ingredient in one compartment and a diluent in another. Multi-component drugs require a division of the EL between each part. This division doesn't have to be equal. For a vial containing 500 mg of active ingredient that requires 10 mL of diluent for reconstitution, one could assign a limit of 0.5 EU/mL (5 EU total) for the diluent container, leaving the balance for the active ingredient: 345 EU per 500 mg dose equals 0.69 EU/mg. This strategy provides a higher, more permissive EL for the active ingredient to allow for more dilution to solve interference problems and more leniency for low-level, safe amounts of endotoxin.

### Multiple Dose Parenterals

The endotoxin limit is based on the maximum amount of drug administered in one hour. One might be tempted to take a labeled total dose of 1.2 g per day and divide by 24 when calculating the EL. However, if the labeling directed dosing at 4 times daily, then the assumption is that all of the divided dose could be given in 1 hour, or 0.3 g per hour. Then, the EL becomes 1.17 EU/mg. An alert limit would be set by taking a fraction of the EL, as described above.

A drug that is administered by slow infusion over a prolonged time represents a different scenario. An example is a cardiovascular drug which has a maximum dose of 0.2 mg per minute for an indefinite period. It is reasonable to base the endotoxin limit calculation on the amount infused during one hour, or 12 mg in this case.

**Summary.** Strategies are presented for calculating endotoxin limits and alert limits for combination drugs, raw materials for multi-ingredient drugs and multi component dosage forms.

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## ROUNDING BET RELATED CALCULATIONS

There are potential pitfalls in rounding off numbers used in calculations associated with the Bacterial Endotoxins Test (BET). Historically, the LAL industry has provided reagents labeled from 0.5 to 0.015 EU/mL. Label-claim assays by LAL producers are derived from Reference Standard Endotoxin in two-fold dilutions from 1 EU/mL. The more sensitive reagents traditionally are labeled 0.06, 0.03 and 0.015 EU/mL, even though theoretically they are 0.0625, 0.03125 and 0.015625 EU/mL. The latter three lambda values are awkward, seem trivial, and imply an accuracy that really isn't applicable to a biological assay that is based on two-fold dilutions.

Why should we be concerned about these untidy lambda values? Although uncommon, there are instances where it is crucial to use the unrounded value. For example, if one uses a 0.03 EU/mL value to calculate release of Water for Injection, a water sample that is positive through a 1:8 dilution yields an endpoint of 0.24 EU/mL. However, use of 0.03125 EU/mL yields a value equal to the 0.25 EU/mL limit. In another example, a 1:16,000 endpoint for an endotoxin indicator assay with 0.06 EU/mL gives 960 EU rather than 1000 EU, if the full factor is used. An MVD calculation where 12.5 EU/mL is divided by 0.06 EU/mL gives 208.33, an overestimation, instead of 200, if 0.0625 EU/mL is applied.

These problems are avoided by writing qualifying statements in the appropriate SOP. Although the FDA-controlled LAL labeling doesn't allow printing the entire lambda, SOPs should direct the use of the full factor for all back-calculations and determinations of BET parameters. After initial calculation with an unrounded lambda, final results should be rounded according to conventional mathematics and the sensible statement in the General Notices section (pp.3-4) of the *USP 23*. Bunnell recently reviewed issues associated with rounding of number in *Pharmaceutical Technology*, p 52-56, May of 1997.

**Annual Charleston LAL Workshops, July, 1998.** This summer's workshops were an outstanding educational and social experience. New topics and lab exercises constituted a truly unique learning opportunity. We thank the participants and instructors who joined us for our 20<sup>th</sup> year LAL Workshops in Charleston.

## CALENDAR

**October 2-3, 1998 - Regulations & Standards Update: Sterilization, Validation and Environmental Monitoring: sponsored by ViroMed Laboratories**

**Seminar Site: The Marquette Hotel, 710 Marquette Ave, Minneapolis, MN.**

**Contact: Mary Ellen Anderson, Director of ViroMed's Tissue Bank Testing Services at 800-582-0077, ext. 224 or by email: [manderson@viomedlabs.com](mailto:manderson@viomedlabs.com).**

**October 14-16, 1998: LAL Workshop for Managers, Hamilton Conference Center in 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.

**Contact: Karen McCullough phone: 908-534-6463; fax: 908-534-1317; email: [karenzm@eclipse.net](mailto:karenzm@eclipse.net). or 908-534-8897.**

**CREL LAL Seminars:**

**Contact Alan Hoffmeister at CREL UK: Tel: 44-1843-822331; fax: 44-1843-822989**

**PDA Annual Meeting: Nov. 9-11, 1998, Washington Hilton & Towers, Washington, D. C.**

**Call PDA for information: 301-986-0293, ext. 131. Visit us at Booth #122.**