LAL UPDATE®

Volume 13, No. 2 **June 1995**

Dear LAL User,

There are now definite signs that summer will soon arrive on Cape Cod. The horseshoe crabs have appeared once again in the shallow bays in preparation for spawning and our laboratory is bursting with activity. Although the amount of our harvest from season to season is difficult to predict, the last two years have seen record yields of LAL and we hope for a repeat this year. Even with increased sales, these good harvests have resulted in an excellent inventory of Pyrotell® (all sensitivities), Pyrotell®-T, and Pyrochrome™.

For our European customers, the changeover from a fragmented distribution system to direct supply and service from Associates of Cape Cod International, Inc. is complete. Our two European offices are located in Walldorf (near Frankfurt) Germany, and Liverpool, England. Our general managers, Dr. Peter Weidner (ACCI-Europe in Germany), and Tony Coyle (ACCI-UK) have extensive experience with all aspects of LAL and endotoxin testing. In addition to our expanded technical services, ACCI-Europe can provide multilingual help for our clients in France, Italy, and Spain.

The Pyros™ for Windows™ software for the LAL-5000 is now firmly established in the marketplace. If you are using the LAL-5000 and have not yet switched to Pyros™, I strongly urge you to consider doing so. It is much easier and faster to use and has a host of advanced features.

As you can see, it is not all vacation and retirement on Cape Cod. Even so, please feel free to make our summer even busier! Our technical service team, Laurie, Keith, and Carmen are ready to answer your technical questions, as are other personnel, including Drs. Dawson and Gould, and of course, myself.

Sincerely,

Thomas J. Novitsky, Ph.D.
President/CEO

Endotoxin Limits

Michael E. Dawson, Ph.D.

It has been established that the pyrogenic threshold for humans is approximately five endotoxin units (EU) per kilogram body weight (1,2). This means that administration of endotoxin concentrations of 5 EU/kg or greater can be expected to elicit a pyrogenic response. Consequently, an endotoxin limit of 5 EU/kg/hr has been set for most injectable (parenteral) products. An injectable product for which this limit applies cannot be sold if the endotoxin concentration is such that a recipient of the maximum dose would receive this amount of endotoxin or more.

The endotoxin limit is represented by the term K. The value of K is 5 EU/kg/hr for injectables, other than those administered intrathecally. For these, the endotoxin limit is more stringent and K= 0.2 EU/kg/hr.

Given that the average human weighs 70 kg, the maximum amount of endotoxin that can be received by a patient being given a non-intrathecal product should not exceed 350 EU per hour (70 x 5 EU/kg/hr).

However, the limit does not account for a patient being given several drugs and/or solutions at

once, which is a common occurrence. Fortunately, most drug products and solutions contain considerably less endotoxin than the allowable limit. Many manufacturers set specifications that are tighter than the required limit.

The calculation of endotoxin limits for injectable drugs administered per square meter is based on the assumption that a typical adult has a surface area of 1.8 square meters. For drugs with a dose expressed per kg, the whole body limit is 350 EU. Thus, the endotoxin limit per square meter is 350 EU/1.8 m^2= 194 EU/ m^2. This is an absolute and is equivalent to a value of 5 EU/kg for *K* for products administered per kg. To calculate the endotoxin limit of a product with a dose expressed per square meter, divide 194 EU/ m^2 by the dose to give an endotoxin limit per unit of product.

Endotoxin limits for radiopharmaceuticals are addressed in a footnote at the end of Appendix E to the FDA Guideline on the LAL Test (3). For these products *K* = 175 (EU)/V (or 14(EU)/V for intrathecals), where V is the maximum (whole body) dose in ml at expiration of the product. The units (the EU given in parentheses) are not specified in the Guideline but must be included if the units are to be expressed in EU/ml.

The USP limit for large volume parenterals (LVPs) is 0.5 EU/ml and is derived from a rabbit dose of 10 ml/kg for Pyrogen tests. A typical LVP administered at 700 ml/hr also gives a limit of 0.5 EU/ml. The USP endotoxin limit for WFI and SWFI is 0.25 EU/ml. For medical devices, the USP limit is 20 EU/device, and 2.15 EU/device for devices that contact the cerebrospinal fluid. The endotoxin limits for devices were discussed in the September 1994 issue of the LAL UPDATE®

Generally, the aim of testing is to insure that the sample under test could not deliver more than 5 (or 0.2) EU/kg/hr of endotoxin to a patient. Thus, the endotoxin limit for a specific product depends on the maximum dose of product that a patient might receive. The smaller the dose of the product per kg, the higher the endotoxin limit per unit dose of the product.

This discussion of limits only applies to release testing of finished products. However, endotoxin limits may be set for raw materials and in-process samples.

Determining the Endotoxin Limit of a Product

The first step is to look up the product in the FDA Guideline to see if an endotoxin limit is listed in the Appendix E. (The most recent revision to Appendix E at this time is dated July, 1994). If the product is listed, check that the maximum dose of the product is correct for your product. If the dose is greater than that given in the table, calculate the limit as described below. It is a good idea to calculate the limit as described below anyway. There have been mistakes in the Appendix in the past.

The 1987 Guideline states that the dose used should be the rabbit dose (as used in the USP Pyrogen Test) or the maximum human dose, whichever is greater. Since 1987, the USP has used only human doses to calculate endotoxin limits and the FDA has incorporated this into their more recent revisions of Appendix E. The term M is used for the maximum dose.

When calculating the limit for drug products, if the dose is expressed for whole (adult) body, first divide it by 70 kg to give the dose per kg. Then divide K (5 EU/kg, 0.2 EU/kg, 194 EU/m^2 or 175 EU) by M (or V) to give the endotoxin limit per unit weight/volume/international unit, etc.

Examples:

Calculation of the endotoxin limit for a non-intrathecal, injectable drug product:

Product A has a maximum human (whole body) dose of 1.0 g

Dose per kg =

$$\frac{1\ g}{70\ kg} = 0.0143\ g/kg = 14.3\ mg/kg$$

Endotoxin limit =

$$\frac{K}{M} = \frac{5\ EU/kg}{14.3\ mg/kg} = 0.35\ EU/mg$$

Use the concentration of the product (potency in final product) to convert the endotoxin limit into EU/ml.

Thus, using the example above, if the concentration of product in the (presentation) container is 100 mg/ml, or if bulk product is dissolved to give 100 mg/ml:

Endotoxin limit =

0.35 EU/mg x 100 mg/ml = 35 EU/ml

Convert the limit to EU/ml because these are the units of the endotoxin standards in the LAL test (as well as the limits for the sensitivity of an LAL gel-clot reagent). This gives an endotoxin limit for the product so that it can be determined whether the product passes or fails at that limit. It is important to note that the limit in EU/ml only applies to the stated concentration of product. If the concentration changes, the limit changes.

The limits calculated above are the maximum allowed. It may be decided that tighter limits are

desirable. Usually the limit is greater than the sensitivity of the test method. If the gel-clot method is used as a pass/fail test, the product must be diluted.

Calculation of the endotoxin limit for a product with a dose expressed per square meter:

For a product with a dose of 1 g/m^2 and a potency of 50 mg/ml:

Endotoxin Limit =

$$\frac{194\ EU/m^2}{1\ g/m^2} = 194\ EU/g = 0.194\ EU/mg$$

$$0.194\ EU/mg \times 50\ mg/ml = 9.7\ EU/ml$$

Calculation of an endotoxin limit for a radio-pharmaceutical product:

For a product with a (whole body) dose at expiration of 7 ml:

Endotoxin Limit =

$$\frac{K}{V} = \frac{175\ EU}{7\ ml} = 25\ EU/ml$$

Conclusion

A clear understanding of endotoxin limits and how they are derived is of critical importance to the release of product by any LAL test method.

References:

1. Elin, R. J., S. M. Wolff, K. P. W. J. McAdam, L. Chedid, and F. Oberling. 1981. Properties of reference *Escherichia coli* endotoxin and its phthalylated derivative in humans. J. Infect. Dis. **144**: 329-336.

2. Hochstein, H. D., E. A. Fitzgerald, F. G. McMachon, and R. Vargas. 1994. Properties of US Standard Endotoxin (EC-5) in human male volunteers. Journal of Endotoxin Research. **1**:52-56.

3. Guideline on Validation of the Limulus Amebocyte Test as an End-product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices. U.S. Department of Health and Human Services, Public Health Services, Food and Drug Administration, December 1987.

Meet Associates of Cape Cod, Inc.'s Technical Services Department

With the continuing expansion of our business, we now have three Technical Services Representatives to answer your LAL-related questions. As in the past, all calls are answered immediately by our Technical Services Department. The department includes: **Laurie Fife**, Senior Technical Services Specialist, **Keith Richardson**, Technical Analyst and **Carmen Barillas**, Technical Service Representative.

Laurie joined ACC in 1993 after doing research for six years in the areas of cell/molecular biology and biochemistry. For two years before moving to Cape Cod, Laurie was a Scientific Liaison at Organogenesis, Inc.

At ACC, Laurie provides technical assistance and participates in the training courses. She specializes in the chromogenic LAL method. She has a BS degree in Animal Science from the University of Vermont.

Keith has been an employee of Associates of Cape Cod, Inc. for more than five years and has experience in many facets of LAL technology, from hemolymph collection to use of our final products. He is responsible for all aspects of turbidimetric LAL testing, and is the technical support person for the LAL-5000 and *Pyros*™ software. As our turbidimetric specialist, Keith participates in ACC training courses. Keith has a BS degree in Biology and Marine Sciences from Southeastern Massachusetts University.

Carmen has an MS in Biology from Worcester Polytechnic Institute and did her thesis work in the Biochemistry Department at the University of Massachusetts Medical Center. She was Senior Research Associate at Genica Pharmaceuticals Corporation, where she did product development and research in molecular biology for two years before joining ACC in 1994. Carmen is a native of Guatemala, Central America, and is fluent in both Spanish and English. The combination of her technical background and language skills makes Carmen a welcome addition to our technical department. She also participates in the training courses offered by ACC.

For expert technical help, regardless of the brand of LAL *Limulus* Amebocyte Lysate you use, call 800-848-3248.

From left to right: Laurie Fife, Keith Richardson and Carmen Barillas