LAL Update
ASSOCIATES OF CAPE COD, INCORPORATED

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Letter From the President

Dear LAL User,

I am pleased to introduce a new edition of the LAL Update and to introduce you to a new Associates of Cape Cod, Inc. (ACC). Since the last edition we have moved into a new, state-of-the-art, 80,000 sq. ft. facility, which has been approved for manufacture of our licensed products. Other changes that have taken place recently at ACC include: a new senior management team that has rededicated the company to quality and customer service; a new, user-friendly website; a new CLIA-approved laboratory for analyzing patient samples for invasive fungal infections; a new Business Development department and a more customer-driven Sales and Marketing department. These improvements enable ACC to develop, manufacture and distribute our products with one driving, underlying goal in mind: to serve the needs of our markets in the most efficient, cost effective and customer-focused manner possible. We will continue to grow, and look forward to highlighting our developments for you in the many years to come.

This issue of the Update discusses one of the most common LAL testing problems; interferences caused by disposable labware. Also included is our calendar of conferences and training courses. Your suggestions on topics of interest for future issues are always welcome, as are customer visits to our new facility.

I wish you a happy, healthy and productive 2005.

Sincerely,

A. J. Meuse, Ph.D.

Laboratory Disposables and the LAL Test

By: ***Laurie A. Fife,***
Technical Services Manager

Michael E. Dawson, Ph.D.
Director of Manufacturing Development

Many LAL testing problems are caused by interferences from laboratory materials. When users are informed of this, our technical support staff often hears that "My materials cannot be the problem. They were labeled non-pyrogenic (or pyrogen-free or sterile)" or, "We have been using the same supplier since …" Unfortunately, suppliers may make changes to their products without notification, or disposables may change over time as they age. Like stocks, a strong history is no guarantee of future performance.

Plastic materials start out as resin (typically beads of the plastic) from which they are manufactured. Molten resin is injected into a mold and then released. The endotoxin burden of the plastic resin is generally low to start with and may be further reduced by the heat of the molding process. To aid in removing the plastic articles from the mold, releasing agents are added. These agents may interfere with the test. The plastics are then packaged and, unless appropriate precautions are taken, may become contaminated during the packaging process. Manufacturers of plastic products for the biopharmaceutical market are generally aware of this and package product under clean conditions.

There are three classes of problem that may be experienced with disposable labware. These are: contamination by endotoxins and/or glucans; leaching of interfering substances, and adsorption of endotoxin. In our own laboratories, over the years, we have identified problems with syringes, pipet tips and different types of plastic tubes. Problems with glass materials are much less common.

LAL Technical Report

Endotoxin contamination of plastic labware is not usually a problem, but it does occur. In tests of plastic tubes from several sources, all extracts from polystyrene tubes were free of detectable endotoxin (<0.001 EU/mL). By contrast, endotoxin (up to 1.5 EU/mL) was detected in all of the extracts from polypropylene tubes, except for one type of tube for which the extract was strongly inhibitory[1]. These results have been well publicized and polypropylene sample or dilution tubes are generally not used in endotoxin testing.

Labels, such as "pyrogen-free, "non-pyrogenic," and "sterile", can be misleading. They may seem to imply that the material is not contaminated with endotoxin. "Pyrogen free" or "Non-pyrogenic" implies that a material will not cause a fever when a human subject is exposed to the extract. However, the endotoxin limit for medical devices is 20 EU/device, and devices meeting this limit are presumed to be non-pyrogenic. This amount of endotoxin is more than enough to interfere with an LAL test. We have seen pipet tips labeled "pyrogen free" which were contaminated with endotoxin. "Sterile" only means that the article is uncontaminated by living organisms. It does not mean that the endotoxin (which can come from living, dead, or even fragments of bacteria) has been removed or neutralized. It is important to understand what these labels mean, how the product is tested and the endotoxin specification limit.

Materials (glass and plastic) may also be contaminated with interfering beta glucans. These will give a positive test result in many LAL tests and also interact synergistically with endotoxin. Associates of Cape Cod, Inc. (ACC) offers a line of disposables, Pyroclear® (see box), which is certified to be free of interfering endotoxin or beta glucans (endotoxin specification: <0.005 EU/mL; glucan specification: <5 pg/mL). This product line includes pipet tips, dilution tubes, reaction tubes and microplates.

The potential for plastics to interfere with the LAL test was recognized by the FDA as far back as 1985 in an Investigators Technical Guide[2]. Materials extracted from plastics and glass can interfere significantly with endotoxin spike recovery. This has been discussed in the literature[1] and in a previous edition of the LAL Update[3]. In our laboratory we have had several experiences with this problem, notably one with plastic syringes, one with polypropylene centrifuge tubes and another with polystyrene test tubes. In all cases, previous batches had shown no such problems. In one instance, a batch of tubes that had been tested and released was not homogenous. An interference problem arose with some of the tubes in the batch which had not been detected in the release test samples.

Different types of plastics may also affect the recovery of endotoxin from disposables. Some plastics, notably polypropylene, are known to adsorb endotoxin from solution. If such plastics are used for sample collection or storage containers, this will result in artificially low test results. Endotoxin recovery from polypropylene tubes has been shown to be very low[4]. This is in sharp contrast with good recoveries from polystyrene tubes and intermediate recoveries from glass. For this reason we recommend against the use of polypropylene containers for sample storage. FDA staff[5] have reported that, contrary to the hypothesis that apparent endotoxin loss from samples might be due to adsorption during storage, inadequate mixing was actually the cause of low recoveries. This awareness of potential sample storage problems suggests that it is prudent to validate sample containers and storage conditions and that appropriate mixing times be specified. It is worth noting that polypropylene pipet tips are commonly used to dispense endotoxin into reaction tubes and plate wells. Adsorption of endotoxin

PYROCLEAR® DISPOSABLE LAL TEST SUPPLIES

Pyrotubes®

TS050	*10 x 75 mm soda lime glass for gel-clot method, 50'pack*
TB050	*10 x 75 mm borosilicate glass for turbidimetric method, 50/pack*
TB240	*12 x 75 mm borosilicate glass (for dilutions), 40/pack*
TB013	*13 x 100 mm borosilicate glass (for dilutions), 18/pack*
TK100	*8 x 75 mm borosilicate glass for Pyros Kinetix, 100/pack*

Pyroplates®

CA961	*96 well microplate, 1 each*

Precision Pipet Tips

PPT25	*250 uL tips, 96 tips/box, 10 boxes/pack*
PPT10	*1000 uL tips, 96 tips/box, 8 boxes/pack*

to the tips is generally not a problem, perhaps because of the short residence time of the liquid in the tip.

The USP Bacterial Endotoxins Test (BET) chapter now includes the stipulation that plastic labware be tested before use. This requirement had been included in the European Pharmacopoeia (EP) and when the USP, EP and Japanese Pharmacopoeia bacterial endotoxins test chapters were harmonized in 2000, it was included in the USP. In USP 28, the chapter states[6], "If employing plastic apparatus, such as microplates and pipet tips for automatic pipetters, use only that which has been shown to be free of detectable endotoxin and not to interfere with the test." A sample of laboratory disposables should be tested before use to determine their acceptability for LAL testing.

In order to test pipets, pipet tips, test tubes or other materials, perform an extraction and then test the extract solution as would be done for a medical device. Take a representative sample of items from the batch to be tested and place them in pyrogen-free water (LAL Reagent Water) at 37°C and extract at room temperature for one hour. This procedure is taken from USP chapter <161>, "Transfusion and Infusion Assemblies and Similar Medical Devices"[7]. Use the minimum volume of water necessary to properly bathe the article. Too much water may dilute contaminant endotoxin below the limit of detection. Test the extract in the normal way with a positive product control (PPC). After testing for endotoxin and interference, vessels intended for sample storage can be tested for sample stability by storing a known concentration of endotoxin in the container for at least the maximum time that a sample will be stored and at the intended storage temperature. Then assay (again with a PPC) the stored sample for endotoxin so that any losses or interference can be determined.

Not only is testing disposable labware for contamination, absence of interference and sample compatibility specified in the USP BET chapter, it is also is prudent and helps avoid unwelcome problems. Consequently, such testing should be part of any LAL testing program, regardless of whether it is conducted in a regulated environment. ACC's Technical Service personnel are always available for consultation on extraction and testing procedures, and our Contract Test Service (see box) is available for development work, validation and routine testing.

References

1. Roslansky, P. F., Dawson, M. E., and Novitsky, T. J. (1991). Plastics, Endotoxins, and the Limulus Amebocyte Lysate Test. J. Parenter. Sci. Technol. 45(2), 83-87.
2. Department of Health, Education, and Welfare, Public Health Service, Food and Drug Administration, ORA/ORO/DEIO/IB. (1985). Bacterial Endotoxins/Pyrogens. Inspectors Technical Guide, No. 40.
3. Anonymous, (1988).The Problems with Plastics, LAL Update, 6(3), 1-3.
4. Novitsky, T. J., J. Schmidt-Gengenbach, and J. F. Remillard, (1987). Factors Affecting the Recovery of Endotoxin Adsorbed to Container Surfaces. J. Parenter. Sci. Technol. 40(6), 284-286.
5. Guilfoyle, D. E., J. F. Yager and S. L. Carito. (1989). The Effect of Refrigeration and Mixing on Detection of Endotoxin in Parenteral Drugs Using the Limulus Amebocyte Lysate (LAL) Test. J. Parenter. Sci. Technol. 43(4), 183-187.
6. Bacterial Endotoxin Test. Chapter <85>. USP 28, United States Pharmacopeia, Rockville, MD.
7. Transfusion and Infusion Assemblies and Similar Medical Devices. Chapter <161>. USP 28, United States Pharmacopeia, Rockville, MD.

CONTRACT TEST SERVICE

Our Contract Test Service (CTS) has extensive experience testing disposables and validating their suitability for use. The CTS will tailor an extraction and testing regime to the needs of each client. A report on the process and results will be provided. To discuss qualifications of disposables or any other testing needs, call CTS at 800-232-5889 or sending an e-mail to testservice@acciusa.com.

LAL News and Events

MARCH

LAL Training Course
March 8-10, 2005
Hilton San Francisco Hotel
San Francisco, CA

LAL Training Course
March 15-17, 2005
Catamaran Resort Hotel
San Diego, CA

Focus on Fungal Infections 15
March 16-18, 2005
Sheraton Bal Harbor
Miami, FL

APRIL

PDA
April 4-8, 2005
Hyatt Regency Hotel
Chicago, IL
Booth: 712

SHEA
April 9-12, 2005
The Century Plaza Hotel
Los Angeles, CA

MTEC Conference
April 15-16, 2005
Hilton Cleveland East Hotel
Cleveland, OH

MAY

ARVO
May 1-5, 2005
Convention Center
Fort Lauderdale, FL

AAMI
May 14-17, 2005
Tampa Convention Centerl
Tampa, FL

LAL Training Course
May 10-12, 2005
HIlton Toronto Hotel
Toronto, Canada

NANT
May 13-16, 2005
Hyatt Regency Hotel
Cincinnati, OH

JUNE

Regenerate
June 1-3, 2005
Westin Peachtree Plaza Hotel
Atlanta, GA
Booth: 104

ASM
June 6-8, 2005
GA World Congress Center
Atlanta, GA
Booth: 912

LAL Training Course
June 7-9, 2005
Falmouth Technology Park
East Falmouth, MA

For more information or to register for a workshop, visit our website at www.acciusa.com
or call (888) 395–2221

Customer Service: custservice@acciusa.com **Technical Service:** techservice@acciusa.com **www.acciusa.com**

ASSOCIATES OF CAPE COD INCORPORATED
124 Bernard E. Saint Jean Drive
East Falmouth, MA 02536–4445

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