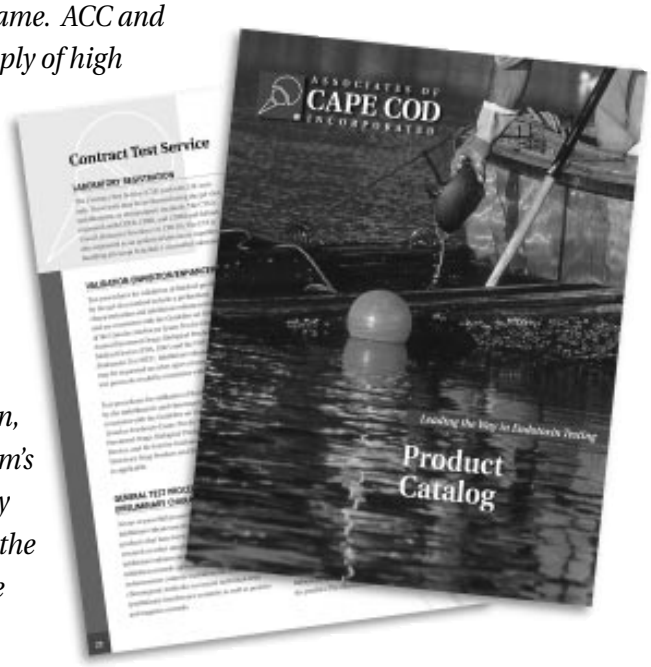
LAL Update®

ASSOCIATES OF CAPE COD INCORPORATED DEC. 1997 / VOL. 15, NO. 4

Letter from the President

As many of you may already know, Associates of Cape Cod, Inc.(ACC) was acquired by Seikagaku Corporation on November 5. All of us at ACC are extremely excited by this event. Seikagaku has clearly established itself as the foremost lysate company in Japan and has a solid track record of innovative technology in the field. Seikagaku was the first company to commercialize the chromogenic LAL test, holds key patents in this area, and was the first company licensed by the USFDA to produce and market a chromogenic lysate made from the Asian horseshoe crab species, Tachypleus. *Seikagaku's Endospecy® was the first endotoxin-specific lysate on the market and they have recently introduced a glucan-specific reagent in Japan. ACC and Seikagaku have already begun the work required to combine product lines and R&D. We are confident that this combination will provide future benefit to our customers by allowing us to expand and improve our products and services worldwide. Aside from additional employees in our sales, technical service, and production departments, the basic structure of ACC in the USA, ACCI in Europe, and Seikagaku in Japan, will remain the same. ACC and Seikagaku have made a long term commitment to a continuous supply of high quality endotoxin and glucan testing reagents, the best technical service available, and a research program designed to improve our understanding of all aspects of LAL, endotoxins and glucans.*



As part of our expanding operation, I am pleased to announce the return of Dr. Malcolm Finkelman. Malcolm was the Director of Product Development for ACC from 1991–1993 and was responsible for moving ACC's Endotoxin Neutralizing Protein product from research to the development phase. Malcolm rejoins us from Procyon, London, Ontario where he was Vice President of Technology. Malcolm's new position at ACC will be Vice President of Clinical and Regulatory Affairs. In this position he will assist with licensing issues related to the new technology from Japan as well as in-house products nearing the end of their development.

Finally, I would like to announce that our new Product Catalog will be available in mid-January. The new catalog will make it easier for both new and old customers to select the ACC products they need. As always, our customer service and technical staff are ready to assist you.

From all of us at ACC and Seikagaku, have a great holiday season and a happy and healthy new year!

Sincerely,

Thomas J. Novitsky, Ph.D.

In This Issue...

SPOTLIGHT

Product Reserve Option

Upon request, Associates of Cape Cod, Inc. will reserve specific lots of product at no charge for a period of up to one year. Product is reserved in accordance with the following terms:

- The amount of product that is placed on reserve is based on the customer's expected usage for a 12 month period or on the customer's purchasing history.
- A purchase order is required to cover the products on reserve. Shipments are made on request and must reference this purchase order.
- At the expiration of the purchase order, any remaining product that has not been purchased will be released for sale.

ACC's Product Reserve Option applies only to Pyrotell,® Pyrotell®-T, Control Standard Endotoxin, Pyrosol,® and LAL Reagent Water.

We Have a Winner!

MARY W. CARVER of Eisai Inc. in Research Triangle Park, NC, has won the "Taste of Cape Cod" dinner for two. The dinner was raffled off at the recent PDA conference in Philadelphia.

TECHNICAL REPORT

Harmonization of Endotoxin Standards and Units

By Michael E. Dawson, Ph.D.

Introduction

The need for endotoxin standards for the LAL test became apparent as soon as the test was developed. A widely used standard in the early years of the LAL test was endotoxin from Escherichia coli *O55:B5 obtained from Difco Laboratories, Detroit, MI. This standard was used in units of mass, usually ng/ml (1 ng = 10^{-9} g). A problem with this approach is that endotoxins differ in their biological activity or potency; the pyrogenicity or LAL reactivity of one endotoxin preparation may be very different to that of an equal mass of another. Recognizing this, the US Food and Drug Administration (FDA) produced a standard* E. coli *endotoxin preparation, EC. Batch EC-2 was assigned units of LAL reactivity, termed endotoxin units (EU). Subsequent lots included EC-5, which has now been succeeded by the current US reference standard endotoxin (RSE), EC-6. This standard is available from the United States Pharmacopeia (USP) as lot G. Expressing endotoxin concentrations in EUs avoids the issues of different potencies of different endotoxins, it also focuses attention on the activity of the endotoxin. Endotoxin potency and standards were discussed in more detail in the LAL Update of September 1993.*

Other national and international bodies have produced their own endotoxin standards and have attempted to relate the potency of these standards to that of the US RSE. However, the question of potency is complicated by the fact that it is an empirical value. Unfortunately, the potency of an endotoxin determined with one LAL reagent lot may differ from that determined with another lot. Consequently, the equivalence of the various international standards has been approximate and this has led to practical problems in the laboratory. The issues of harmonization between the US, the World Health Organization and the European Pharmacopoeia have now been resolved by the adoption of a common standard. While the various authorities have given different names and lot numbers to the standard, the content of the vials is identical and the units are truly equivalent.

The Harmonized Standard

The harmonized standard consists of endotoxin extracted from the cells of *E. coli* O113, with polyethylene glycol and lactose fillers. It is assigned an activity of 10,000 Endotoxin Units (EU) or 10,000 International Units (IU) per vial. The standard was prepared for USFDA, the USP and WHO by Dr. Stephen Poole at the National Institute for Biological Standards and Control in the UK. This standard was accepted by the USFDA as lot EC-6 and by the USP as lot G. The potency was determined to be 10,000 EU/vial in collaborative studies referencing EC-5/lot F.

The WHO has adopted this endotoxin preparation as the Second International Standard (IS). Poole *et al*., (1997) report on the calibration of the second IS and state: "…the candidate standard is suitable to serve as the second international standard for endotoxin for all applications, with an assigned unitage of 10,000 international units (IU)/vial, with 1 IU = 1 EU (FDA/USP endotoxin unit)." The authors recommended to the Expert Committee on Biological Standardization of the

WHO that this standard be adopted. This was accomplished at its meeting 8–11 October, 1996 (S. Poole, personal communication).

The European Pharmacopoeia (EP) has accepted the standard as the third Biological Reference Preparation for endotoxin (BRP-3). BRP-3 replaced BRP-2 in September of this year, but the change was not been formally announced. Changes in BRP lots are not routinely publicized. However, an announcement is planned for the December issue of PHARMEUROPA (E. Charton, personal communication). The announcement will state that BRP-3 is part of the batch established as the 2nd IS by the WHO and that the FDA and USP standards are from the same batch. Both the WHO and EP have labeled vials of the standard as containing 10,000 IU. Consequently, the harmonized standard has four different lot designations and two different units of activity, but it is one standard and, most importantly, 1 IU = 1 EU.

Practical Considerations

The key question for most readers is "what is the impact of harmonization upon LAL testing?" The answer is a positive one, and it is surely cause for rejoicing when changes at the regulatory level actually make life simpler in the laboratory. Thanks to the common standard, results in EU can equally be reported in IU and vice versa. Associates of Cape Cod's Certificates of Analysis now give CSE potencies in EU/ng and IU/ng. Potency differences between BRP-2 and the RSE will no longer cause problems for LAL users, now that BRP-2 has been replaced by BRP-3.

The primary reference for the equivalence of units is the paper of Poole et al., (1997). The USP accepts the equivalence of the IU to the EU for endotoxin. The matter is addressed in the General Notices of the USP (page 5) in the section on Units of Potency. This states "Units of biological potency defined by World Health Organization (WHO) for International Biological Standards and International Biological Reference Preparations are termed International Units (IU). Units defined by USP Reference Standards are USP Units, and the individual monographs refer to these. Unless otherwise indicated, USP Units are equivalent to the corresponding International Units, where such exist. Such equivalence is usually established on the basis solely of the compendial assay for the substance."

The final word on this topic is left to Poole et al., (1997), who close their paper with the words "…1 IU = 1 EU, avoiding the need to convert one into the other and achieving the global harmonization of unitage for standards of endotoxin."

References

"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." U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration, December, 1987.

LAL Update ***11****(3), September, 1993.*

Poole, S., P. Dawson and R. E. Gaines. *1997. Second international standard for endotoxin: calibration in an international collaborative study. Journal of Endotoxin Research.* ***4****(3): 221–231.*

United States Pharmacopeia–National Formulary, 23 Revision, 1995, U.S. Pharmacopeial Convention, Rockville, MD, 1994.

Associates of Cape Cod, Inc.'s Information Systems and Year 2000 Compliance

Associates of Cape Cod, Inc. depends on its computerized systems to supply materials to its customers as well as to track product/customer/company information. To this end ACC has for the past several years specified that all computers, operating systems, software, and other related sub-components be Year 2000 compliant.

ACC's computerized order entry, order processing, invoicing, billing, and all other accounting functions are performed using Solomon Software's (Findlay, Ohio) Solomon IV for Windows. Solomon Technical Bulletin 4092 entitled Solomon Software and the Year 2000 states: "Solomon IV for Windows products have been designed to accurately store dates correctly for the 20th century (1900s) and the 21st century (2000s)."

Solomon Software at ACC is executed on Microsoft's Windows 95 client computers connected to network servers running Microsoft's Windows NT server operating system. The date limits specified for Win95 and Windows NT are 2108 and "future centuries" respectively (Microsoft's web page @ http://www.microsoft.com/cio/articles/year2000).

In addition, all critical networked computers executing Solomon utilize American Megatrends BIOS which as of BIOS date 7/15/95 are all 2000 compliant (American Megatrends web page @ http://www.amibios.com/2000).

ACC thus anticipates no interruption of goods and services, or loss of customer/company information, during the transition to the year 2000.

Pyros® and the Year 2000

Pyros (current version 1.02) is a Windows® program and is Year 2000 compliant. The program is not date critical but does take a date stamp from the operating system, as recommended by Microsoft. MS-DOS, Windows and Windows 95 all use four characters for the year and so are not sensitive to the change from 1999 to 2000.

Pyros does include an autofilename option which offers a default filename consisting of a six character date (mmddyy, ddmmyy or yymmdd, format selectable by the user) followed by a dash and a letter, starting with A for the first file of the day. When the computer clock changes to the year 2000, the yy will read 00. However, every file is also stamped with the full date with four digits for the year (see **View**, **Test Info**. in either the **Collect Data** or **Analyze Data** modules and any printout).

Pyros was tested by changing the date on the clock of the PC on which it was running. The clock was changed so that a test was initiated in 1999 and then the year changed to 2000 while the test was running. The data file is stamped with the date on which the file was closed. This test showed that files are correctly stamped with the date.

Pyros was also tested for tests initiated in the year 2000, again without any problems. The same testing will be carried out on future releases of the program. Problems are not expected because the operating system has dealt with this matter.

New Water Packaging

ACC announces the arrival of new packaging for LAL Reagent Water (LRW). Customers who purchase multiple 50 ml or 100 ml bottles of LRW will notice that the water is now packaged in a cardboard box specially designed to prevent breakage.

ACC is also offering volume packaging of the water at a cost savings. We now offer our 50 ml LRW in a convenient 5 pack and our 100 ml LRW bottles in 4 packs.

Catalog#	Description
W0051	5.5 ml/bottle
W0504	50 ml/bottle
W050P	50 ml/bottle, 5 bottles/pk – **NEW**
W1004	100 ml/bottle
W100P	100 ml/bottle, 4 bottles/pk – **NEW**

Please call our Customer Service Department if you would like pricing on the new LRW packaging.

ASSOCIATES OF CAPE COD INCORPORATED

Creating New Horizons in Endotoxin Testing

704 Main Street ▪ Falmouth, MA 02540

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For customer service call (800) LAL–TEST or (508) 540–3444. For technical service call (800) 848–3248 or (508) 540–3444.
Please visit us on the internet at www.acciusa.com.