LAL UPDATE®

# International Edition

**Volume 12, No. 1** **March 1994**

*Dear LAL User,*

*As Associates of Cape Cod enters its 20th year in the LAL business, I would like to take this opportunity to thank our capable and dedicated employees, hard working distributors, and particularly, our loyal customers. Although this UPDATE reviews our history and past successes, we at ACC are far more interested in the future. Plans for the coming years include new and improved LAL products that will be developed in an upgraded facility. Since ACC is the world's leading supplier of LAL, we have embarked on the road to ISO 9000 certification, as have our major European distributors. An increase in the size of our technical staff and number of workshops will assure our continuation as the "LAL Knowledge Leader". This fall we will introduce our Windows™ version LAL software to coincide with the availability of the new US made LAL-5000 Automatic Endotoxin Detection System.*

*Associates was founded on quality products and expert technical assistance. On this our twentieth anniversary, we have reaffirmed our dedication to these principles and are convinced they will assure our success through the next twenty years and beyond.*

*Sincerely,*

*Thomas J. Novitsky, Ph.D.*
*Editor*

# The History of LAL and Associates of Cape Cod, Inc.

This year, 1994, Associates of Cape Cod celebrates its twentieth anniversary. It has been 20 years of growth, from a staff of 5 working in a basement to 45 full-time employees in a fully modernized facility.

Associates of Cape Cod, Inc. (ACC) was founded by Dr. Stanley W. Watson in 1974 to produce *Limulus* amebocyte lysate (LAL) for the detection of endotoxin, a cell wall component of gram-negative bacteria.

**Discovery of LAL**

In the 1950's Frederik Bang, a Johns Hopkins researcher working at the Marine Biological Laboratory in Woods Hole, MA, made a remarkable discovery. He found that certain kinds of bacteria injected into the bloodstream of healthy horseshoe crabs caused massive intravascular clotting. Later, Dr. Bang, in collaboration with another Hopkins scientist, Dr. Jack Levin, showed that the causative agent was actually bacterial endotoxin, and that a clot formed in a test tube when endotoxin was added to an extract of the horseshoe crab blood cells or amebocytes. Bang and Levin determined that the reaction is enzymatic and that the enzymes are located in the granules of the amebocytes. According to current understanding, the reaction leading to clot formation is a non-reversible cascade of enzyme activation steps resulting in activation of the clotting en-

zyme. Clotting protein or coagulogen is cleaved by the activated clotting enzyme and the insoluble cleavage products coalesce by ionic interaction to form the gel matrix.

**Early Beginnings**

The beginnings of Associates of Cape Cod can be traced back to the early 1970's when Stanley Watson, Ph.D., a scientist at the Woods Hole Oceanographic Institution (WHOI), was trying to purify certain membrane components from some gram-negative marine nitrifying bacteria. The LAL reagent appeared to be a convenient way of assessing the purity of these components, since lack of endotoxin would signify the absence of cell-wall material. Unfortunately, LAL was in short supply, and not sensitive enough to detect the very small amounts of endotoxin needed to assess the purity of membrane preparations. Dr. Watson decided to make his own LAL, and in the process improved the sensitivity of the reagent. This effort soon grew into a major research project. In 1973, Watson's group was beginning to turn out good quality, highly sensitive LAL. In 1974, armed with a patent for making highly sensitive LAL, Dr. Watson founded Associates of Cape Cod, Inc.

Those days were trying but fun. Crabs were bled on the lawn or in the garage while space was somehow made for laminar flow hoods, depyrogenating ovens, and an autoclave in the already cramped basement. During these early years, the sensitivity and stability of the reagent continued to improve. Dr. Watson worked with the Bureau of Biologics on the regulatory aspects of LAL. Soon both activities paid off and in 1977 the FDA granted ACC the first product and facility license for LAL. This same year ACC said farewell to Dr. Watson's basement and moved 4 miles to a leased facility on Rose Morin Drive in the town of Falmouth, MA. Because of its strong ties to WHOI and the Woods Hole area, ACC has retained its mailing address in the small Woods Hole Post Office.

**A Young Company Grows Up**

Starting with just seven customers in 1974, the company has continued to grow and the customer base now numbers in the thousands. One remarkable feature of this 20 year history is that the first product, the 5 ml 50 test vial of LAL that is still the key to the catalog, has not changed in price since the beginning. The widely used single test vials and 1 ml and 2 ml fill sizes were all approved by the FDA in 1978.

The 1980's were an exciting period of growth as pharmaceutical companies, encouraged by a number of far sighted individuals at the FDA, moved from the use of rabbits for pyrogen detection to LAL. It was not until the early 1990's that the USP monographs changed from specifying the Pyrogen (rabbit) Test to the Bacterial Endotoxins (LAL) Test. The 1980's also saw a dramatic growth of interest in the use of LAL abroad. While replacement of the pyrogen test by LAL has not proceeded as rapidly as in the USA, the use of LAL for in-process testing has increased dramatically.

The rapidly expanding company soon outgrew the facility occupied since 1977. In 1982 Dr. Watson purchased the current building on Main Street in Falmouth and, after extensive remodelling, began production. Even this much larger facility proved constricting and following acquisition of an adjacent lot, a new wing was added and commissioned in 1991.

**Innovations**

A major innovation by ACC was the introduction of a kinetic turbidimetric LAL assay system in the early 1980's. The first commercial kinetic LAL assay consisted of a specially formulated LAL reagent, Pyrotell-GT®, which could be used for the gel-clot and turbidimetric assays. The kinetic assay also required an incubating optical reader in which to perform the test and software to collect and analyze data. After a cooperative venture with Abbott and their MS-2 instrument, which already performed many of the functions required, the LAL-4000 was introduced in 1984. The LAL-4000 was developed in conjunction with Benthos, a deep sea engineering company that had also grown out of the oceanographic activities in Woods Hole. The LAL-4000 was the first instrument specifically designed to perform LAL tests and, in conjunction with a new turbidimetric reagent, Pyrotell-T® increased the sensitivity of the LAL test by at least a factor of ten with a detection limit of 0.001 EU/ml, a limit that still stands. The LAL-4000 was replaced by the LAL-5000 in 1987. The new instrument offered increased sample capacity, reliability and ease of service. These features were further enhanced in 1990 by the LAL-5000 *Series 2*, which was built in Europe. The new instrument was coupled with improved software which was upgraded in 1989. Ten years after the advent of the LAL-4000, the success of the LAL-5000 continues. It is again being produced in the USA, and will be released this fall with a completely rewritten Windows™ software package complete with powerful enhancements.

In 1985, ACC decided to pursue some non-LAL related research on the horseshoe crab. This resulted in the discovery of an endotoxin neutralizing protein (ENP). Because of its high specificity and tenacity for endotoxin, ENP formed the basis of a new line of endotoxin removal products, END-X™, and has also been the subject of many preclinical studies to assess its potential as an anti-sepsis therapeutic drug. Studies of ENP continue with the hope that someday this compound may become an important product for ACC.

Our most recent product, the single-

step chromogenic reagent, Pyrochrome™, was introduced in 1993. This followed research and collaboration with our distributor in Japan, Seikagaku Corporation, the original patent holders for the chromogenic LAL test. In the new reagent, LAL and the chromogenic substrate are co-lyophilized in the same vial which increases the ease of use and flexibility. The same reagent is used in both endpoint and kinetic methods. Also available is the diazo-coupled endpoint modification. This results in a shift in color from yellow to magenta. It is particularly useful for samples that absorb in the yellow range, including biological and clinical samples. The diazo-coupling method is the foundation of the SepTest™ kit that is being evaluated as a diagnostic for the detection of endotoxin in blood. It would indeed be fitting if the first company licensed to produce LAL, and one born in Woods Hole where LAL was discovered, should produce a diagnostic test kit for blood endotoxin and so realize the potential Levin and Bang saw for this remarkable *Limulus* reaction 40 years ago.

The story of Associates of Cape Cod and the development of LAL for the detection of endotoxin is one example of the unexpected result of truly basic research. Drs. Frederik Bang and Jack Levin were engaged in research for which there was no apparent commercial application. With the help of Dr.Watson, their discovery has become a great benefit for everyone.

**1974 - 1994**

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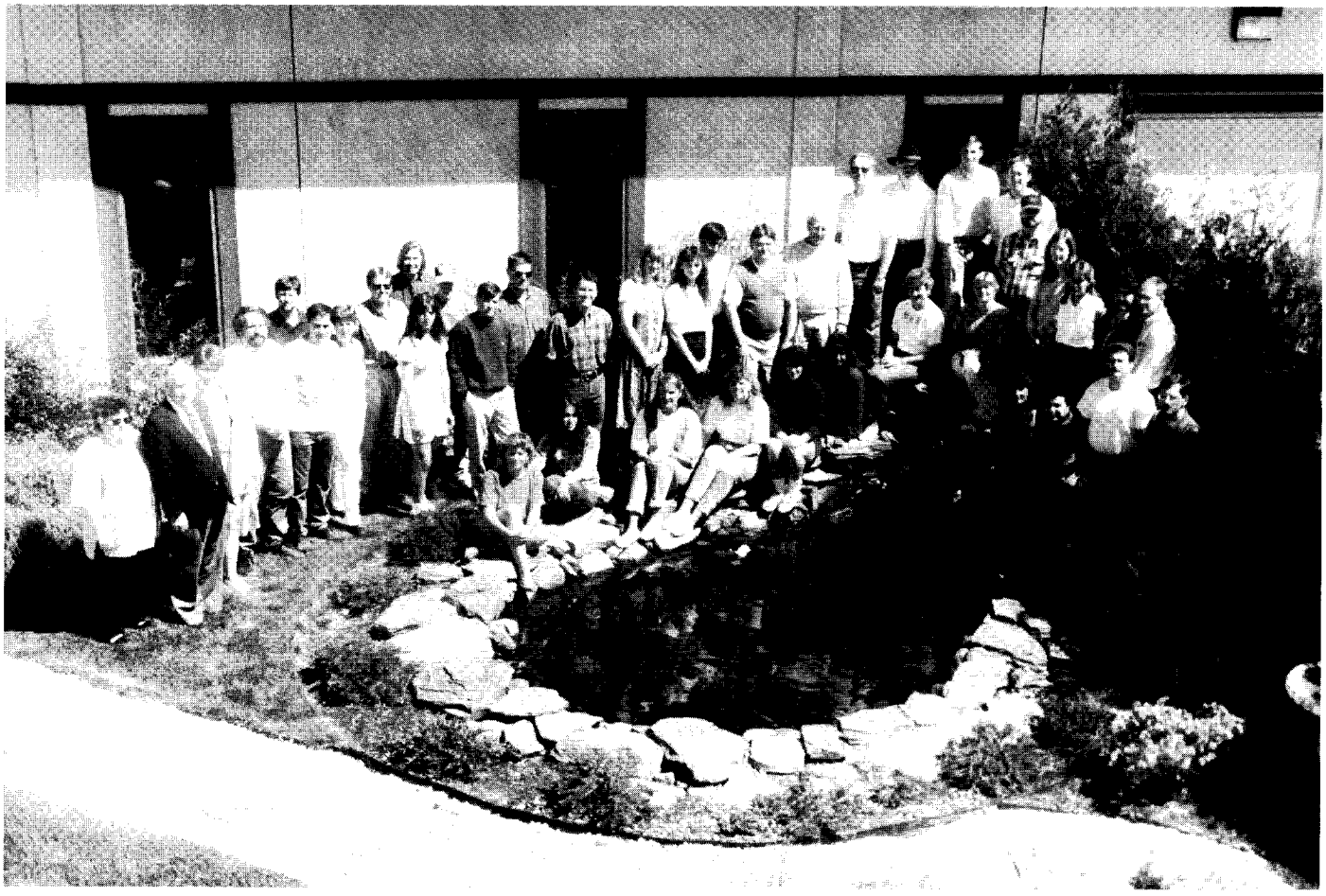
**YEARS OF EXCELLENCE**

**Associates of Cape Cod,Inc.**



**Employees of Associates of Cape Cod, Inc.**
**June 1994**