

Letter From the President



What better way to start the new millenium than to improve on a standing success, the LAL 5000.

While the basic concepts of depyrogenated glass tubes, precise temperature control, at-will test addition, and consistent 0.001 EU/ml sensitivity still provide tremendous advantages over the microplate LAL method, there is always room for improvement. Therefore, while maintaining the basics, we have redesigned the LAL 5000 to accommodate 96 tubes (in a single unit), allow a 1:1 sample to LAL ratio to overcome inhibition in difficult samples, and have upgraded the accompanying Pyros software to be compatible with Windows 95, 98 and NT. In addition, the new instrument, dubbed the Pyros Kinetix, is now completely solid state and uses less expensive 8 x 75 mm glass tubes.

As of June, Pyros Kinetix units began shipping to customers. This Update describes all the exciting features of the Pyros Kinetix. Subsequent issues of the LAL Update will showcase the advantages of using the Pyros Kinetix with emphasis on glass vs. plastic, product inhibition, reproducibility, and cost savings.

Sincerely,



Thomas J. Novitsky, Ph.D.

Pyros® Kinetix

by Thomas J. Novitsky, Ph.D

Since Jack Levin described the "kinetics of clot formation" in 1968 only two companies have actively promoted a "turbidimetric" LAL methodology, Worthington, Inc. (no longer in the LAL business) and Associates of Cape Cod, Inc. Worthington's reagent was used in an end-point assay as was our original research-use-only reagent. It became readily apparent that an end-point assay was difficult to perform, limited in sensitivity range, and nearly impossible to scale-up (i.e. perform many samples at one time). The introduction of Pyrotell-GT kinetic turbidimetric reagent and the LAL 4000 changed this and was an excellent competitor for the end-point chromogenic assay introduced around the same time. The evolution of Pyrotell-T and the LAL 5000 helped ACC remain competitive. However, with the advent of a kinetic chromogenic reagent, improved microplate quality (less frequent well contamination), and microplate readers with reasonable temperature control, the 32-well LAL 5000 finally met some serious competition. The use of two additional modules to give a total of 96 wells partially addressed the capacity issue. In addition, while the LAL 5000 system still boasted the most sensitive LAL assay available, 0.001 EU/ml, some LAL users were swayed by the argument that the 1:1 LAL to sample ratios in the microplate method (compared to the 1:4 LAL to sample ratio used in the LAL 5000) reduced the amount of dilution needed to overcome inhibition.

Therefore, when contemplating the design of the next generation LAL 5000, we decided that in addition to conserving all that was great with the LAL 5000, the new version would have a 96 tube capacity (similar to a microplate reader), and would read a 1:1 LAL to sample test with no increase in the amount of reagent needed. In addition, we decided to see if we could keep the cost competitive with microplate readers (instrument as well as disposables), and upgrade the software.

I am proud to say, we accomplished all of the above and more.

The Pyros Kinetix is a 96 well single unit with an 18 inch linear footprint. Thus Pyros Kinetix takes up the same bench space as a typical incubating microplate reader while having identical sample capacity. However, Pyros Kinetix uses inexpensive 8 x 75 mm borosilicate glass tubes that are depyrogenated in a validated process (vs. plastic microplates which cannot be depyrogenated). Because glass tubes are dry-heat depyrogenated and are handled individually for testing (make a mistake and only one tube is wasted, not an entire plate), the likelihood of an out-of-specification result due to inadvertent contamination is greatly reduced. The smaller 8 x 75 mm tubes allow use of a 1:1 LAL (0.1 ml) to sample (0.1 ml) ratio, if needed, to reduce dilution of highly inhibitory samples.



In addition, the smaller tube size means less LAL is used, resulting in a savings on reagent. Since the smaller 8 x 75 mm tube requires less total volume for the test to be read by the Pyros Kinetix, the user now has the option of 0.1 ml LAL and 0.1 ml sample (1:1 ratio) or 0.05 ml LAL and 0.2 ml sample (1:4 ratio). The later, obviously saves LAL. Since existing LAL-5000 users are already successfully using a 1:4 ratio, switching to the Pyros Kinetix will instantly result in a 50% saving in their reagent cost, easily justifying the cost of instrument replacement. For microplate users, additional savings using the 1:4 ratio with Pyros Kinetix should more than offset any benefit realized from their current 1:1 methodology. In addition, microplate users benefit from increased sensitivity with Pyros Kinetix (0.001 vs 0.05). ACC is committed to reducing the amount of LAL reagent used in order to help conserve the horseshoe crab population. We hope LAL users will join us in this effort.



Finally, the revised Pyros software includes Windows 95/98/NT compatibility, real time temperature display, dynamic editing, rapid set-up and format recall, data export for LIMS and trending, raw data storage in the event of power failure, and security options. Some time next year, a new version of Pyros will be available, which will fully comply with 21 CFR 820.30 Design Controls, the current FDA's General Principles of Software Validation (last available draft at this writing was June 1997), and 21 CFR part 11 Electronic Records; Electronic Signatures. It will also have multi-language text options (English, Spanish, French, German, and Italian), and built-in trend analysis.

Pyros® Upgrade

There are two new releases of the Pyros software:

Pyros version 1.05 is the final upgrade for Windows 3.1 and is available free-of-charge to customers with older versions of the Pyros software. The upgrade repairs a few minor quirks in the older version of the software and will be the final revision of Pyros for the Windows 3.1 operating system. The program is only available on 3.5" floppy disks. To receive your free upgrade you will need to supply the serial number of your LAL-5000 Master Module and the version of Pyros you are currently using. Requests can be made by telephone to our customer service department at (800) LAL-TEST or can be submitted from our website. There may be a nominal charge for shipping depending on your location and the mode of delivery chosen.

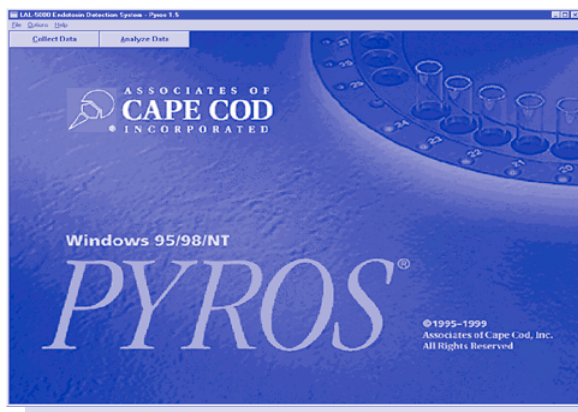
Pyros version 1.5 is also available for the LAL-5000. It is the first 32-bit version of the Pyros program. This version is designed to run on Windows 95/98/NT and has the advantage of allowing longer file names (up to 80 characters). NT is increasingly popular in GMP regulated facilities because of its stability and security features. Future upgrades are planned for this software including multi-language capabilities, trending, and audit trail capabilities. We

expect to have a 21 CFR part 11 compliant software package available sometime in the second half of 2001. Pyros 1.5 is only available on CD-rom.

Through the end of the year, Pyros version 1.5 will be available at a significant discount.

Please call our Customer Service Department at (800) LAL-TEST for pricing or call your local Distributor.

You can also email your request for pricing to custservice@acciusa.com.



State of the Horseshoe Crab

According to environmentalists, the horseshoe crab is in trouble—not from the LAL industry, as we have been one of the few groups looking out for the welfare of *Limulus* since the FDA licensed LAL in 1977, but from increasing use of the horseshoe crab as bait, in commercial fishing. Unfortunately, all pressures on the horseshoe crab affect the LAL industry. The latest and greatest of which is the dramatically increasing use of this ancient animal as bait for conch (whelk) and, to a lesser extent, eel. Although ACC's supply of crabs for LAL is for the moment more than adequate to meet industry needs, we cannot predict the future. For the past two years ACC has worked with various organizations to insure wise use of the horseshoe crab. Our major effort has been with the Atlantic States Marine Fisheries Commission (ASMFC) which earlier this year adopted limits on the taking of horseshoe crabs as bait. Fortunately, due to the participation of ACC and other LAL manufacturers and the great track record the LAL industry has in returning crabs alive to their natural habitat, the ASMFC's regulations allow exemptions for the LAL industry. Unfortunately, the US Department of Interior did not agree with our record nor the LAL industry's as a whole even though their sister agency the FDA has been the only federal group that was concerned about the horseshoe crab's welfare from the late '70s until now. Interior's new found concern for the crab resulted in a closing of traditional fishing areas in Massachusetts and elsewhere. Therefore ACC sued the Department of Interior, specifically the Cape Cod National Seashore and the Monomoy National Wildlife Refuge, areas on Cape Cod where we have collected crabs for over 20 years. We were rewarded for our efforts by being granted a preliminary injunction in Federal District court (Massachusetts) on May 18, 2000, and have been conducting business-as-usual since. Fortunately the injunction came just in time for the start of horseshoe crab bleeding season. We continue to be vigilant, however, as it became apparent during the hearing that the Department of Interior did not fully appreciate the importance of LAL to the Nation's pharmaceutical industry and to its public health.

Federal closures are also predicted or actually already occurring in other areas along the East Coast, especially in the Delaware Bay (primarily New Jersey and Maryland).

In an effort to reduce taking of horseshoe crabs by bait fisherman, ACC has been conducting research on alternative conch/eel bait for the past ten years. This research has resulted in a bait candidate that will be extensively field tested this season. The bait is actually a by-product of LAL production, and although ACC has a patent-pending on this material, we have decided to provide a free license and know-how to anyone willing to provide this alternative to fishermen at cost.

Finally, ACC has been actively working on an alternative assay for endotoxin that does not require the horseshoe crab. This research is now close to the point where we can consider applying for FDA approval.

Further Reading

1. **American Bird Conservancy.** 1999. Horseshoe Crab Harvest Threatens Shorebirds: Plan Pending. *Bird Calls*. 3:9.
2. **Berger, T.** 2000. Horseshoe Crab Management Board Approves State Plans to Reduce Coastwide Commercial Bait Landings by 25%. News Release, Atlantic States Marine Fisheries Commission, April 4, 2000, 1444 Eye Street, N.W., 6th floor, Washington, D.C. 20005.
3. **Berkson, J.** and C.N. Shuster, Jr. 1999. Fisheries Management - The Horseshoe Crab: The Battle for a True Multiple-Use Resource. *Fisheries* 24:6-10.
4. **Fair, J.** 2000. Regional plan for horseshoe crab conservation. DMF News (Massachusetts Department of Marine Fisheries), First Quarter 2000, p. 5-6.
5. **Manion, M.M., R.A. West,** and R.E. Unsworth. 2000. Economic Assessment of the Atlantic Coast Horseshoe Crab Fishery. Prepared by Industrial Economics, Inc., 2067 Massachusetts Ave., Cambridge, MA 02140, April 7, 2000, for the Division of Economics, U.S. Fish and Wildlife Service, 4401 N. Fairfax Drive, Arlington, VA 22203.
6. **Rudloe, A.** 1983. The Effect of Heavy Bleeding on Mortality of the Horseshoe Crab, *Limulus polyphemus*, in the Natural Environment. *J. Invertebrate Pathol.* 42:167-176.

CALENDAR OF EVENTS



SEPTEMBER

September 13 - 15

LAL Methodology and Applications Seminar and Workshop

Westin Harbor Island Hotel

San Diego, CA

September 19-21

LAL Seminar and Workshop

Woolton Redbourne Hotel

Liverpool, England

For additional information and registration, please call our U.K. office at +44 0151-220-3336

OCTOBER

October 10

Advanced LAL Discussion

Woolton Redbourne Hotel

Liverpool, England

For additional information and registration, please call our U.K. office at +44 0151-220-3336

DECEMBER

December 4 - December 7

PDA Annual Meeting

Philadelphia Convention Center

Booth # 123 & 125

December 11 - December 15

ASCB

Moscone Convention Center

Booth # 1222

San Francisco, CA

For customer service:

call (800) LAL-TEST or (508) 540-3444.

For technical service:

call (800) 848-3248 or (508) 540-3444.

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