Charles River ENDOSAFE

LAL TIMES

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## *EDITOR'S COMMENTS*

*New Product: It is a common practice in the industry to store endotoxin dilutions for multiple days. This routine is fraught with container interference, endotoxin aggregation and concern for microbial contamination. The focus of this issue is a new CSE concept that combines the advantage of a stabilized endotoxin dilution series with regulatory compliance. The new system also endorses the application of "hot spikes" to prepare PPCs - one of the important techniques adopted in the industry during the past decade.*

*Year 2000: As the new millennium approaches, it is crucial to investigate the Y2K compatibility of software used in the LAL lab. Charles River has addressed this concern relative to the BioliseÔ kinetic LAL software.*

***Dr. James F. Cooper***

# Extended CSE Dilution Kit, CXE™

F.T. Jordan and J.F. Cooper

**Introduction:** Daily preparation of the SDS (standard dilution series), 2λ through 0.25λ , is one of the most tedious parts of routine BET tasks. It is the most frequent source of variation in LAL-test laboratories. In the last issue (***LAL Times***, December 1998), we reviewed causes of variation associated with the SDS and focused on proper use of control standard endotoxin (CSE). This discussion describes a way to minimize time-consuming vortex mixing and dilution.

**New CSE Product**: A new type of CSE is available from Charles River Endosafe to eliminate the problems associated with daily preparation and storage of the standard dilution series. The Extended CSE Dilution Kit, **CXE**™ , is introduced to simplify and assure the maintenance of reliable standards. It is the first product of this type that meets regulatory requirements. Endosafe certifies that the components will perform as described in the labeling. The kit greatly reduces time spent in preparation of standards while it maximizes their reliability in routine use.

It provides a scheme for long-term use and storage of endotoxin standards that is consistent with the Bacterial Endotoxins Test (BET). Most important, it assures daily verification of label claim and correct preparation of positive product controls (PPCs). Finally, it provides sufficient volume to permit repeated usage or retests of suspicious data.

**Description of Product:** The kit contains one 50-mL vial of Endotoxin Stabilizing Diluent for making stable CSE solutions. The diluent is LAL Reagent Water which has low concentrations of an endotoxin dispersing agent and bacteriostatic agent. The kit also contains a package of depyrogenated, 16 x 90 mm dilution tubes with convenient screw caps. Finally, there is a vial of CSE with a certificate of analysis (CoA). The tray in the packaging becomes a test-tube rack for storage of standards which have long-term stability.

**How to use CXE**™ : The new CSE is rehydrated with the stabilizing diluent as directed by the CoA. Subsequent dilutions are also made with the stabilizing diluent. The directions describe how to make the 20-lambda tube, for preparation of PPCs, as well as the standard dilution series (2λ through ¼ λ ). The entire set of solutions has a certified 4-week stability at 2-28 ° C. Subsequent use of the standards simply require vortex mixing of the standards for 1 minute prior to conducting the daily verification of label claim, done with the first set of tests.

**Supporting data**: A representative stability study is presented in Table I which demonstrates the reproducibility of the Extended CSE Dilution Kit over a 5-week period. The study was initiated by preparing the SDS with the stabilizing diluent and making the first analysis. Then, subsequent analyses were completed at weekly intervals. The data was generated in triplicate on a Toxinometer ET-201, a kinetic tube-reader which collects gel-clot and kinetic turbidimetric analyses (KTA) simultaneously. Table I gives the gel-clot results and KTA reaction times in minutes. Reaction times vary inversely with endotoxin strength and are a sensitive measure of standard potency. During the course of the study label claim was consistently confirmed, and there was no significant difference among the reaction times of each endotoxin concentration.

Table I. STABILITY OF STABILIZED CSE BY GEL-CLOT AND KTA METHODS

STANDARD DILUTION SERIES

| *Time (week)* | *2λ* | λ | ½ λ | ¼ λ |
|---|---|---|---|---|
| 0 | + 31.2 | + 39.8 | - 49.5 | - >59.8 |
| 1 | + 32.0 | + 37.0 | - 47.4 | - >59.8 |
| 2 | + 30.1 | + 36.3 | - 46.3 | - >59.8 |
| 3 | + 32.3 | + 39.5 | - 46.0 | - >59.8 |
| 4 | + 32.3 | + 37.7 | - 48.1 | - >59.8 |
| 5 | + 29.5 | + 34.6 | - 53.2 | - >59.8 |

**Product certification**: A Certificate of Analysis certifies that the RSE/CSE determination was done in accordance with the BET of the **US Pharmacopeia**. The CoA specifies the LAL reagent and stabilized CSE used in the ratio determination. The CoA provides the endpoint of the analysis in ng/mL and gives the ratio of the dried endotoxin in EU/ng, as required by the BET and Appendix C of the FDA's LAL-test Guideline.

**Regulatory issues**

Other attempts by the industry to introduce endotoxin standards with long-term storage characteristics have not met pharmacopeial requirements; thus, these approaches are non-compliant with Good Manufacturing Practices. For example, the FDA's Guideline and BET require an end-point dilution series to prove that the PPCs are made at proper strength and verify that analyst proficiency and analysis conditions are in control. Without an end-point confirmation of the strength of the spiking solution used to prepare the PPCs, the procedure does not comply with the BET. In contrast, the **CXE**™ kit allows for endpoint confirmation of all solutions used in routine testing.

**Hot Spike Technique for PPCs**

During the past decade, the "hot spike" method for preparing PPCs has proven to be the most accurate, reliable and convenient way to make this crucially important control. It simplifies PPC preparation by eliminating a separate tube for the PPC. Further, it avoids inhibitory conditions caused by exposure of the endotoxin spike to drugs, such as ketamine, which reduce endotoxin activity after 20-30 minutes (mechanism unknown). Inoculation of the diluted product or extract by this technique shortly before addition of LAL reagent is the most reliable way to prepare and recover PPCs in both gel-clot and kinetic LAL methods.

The **CXE Kit**™ embodies the "hot spike" technique of preparing positive product controls *by spiking 10 microliters of 20λ into a tube of test sample*. The 20λ spiking solution is a part of the dilution scheme. Whether or not **CXE**™ is used, all test facilities are encouraged to modernize their SOPs and adopt the "hot spiking" method for PPC preparation.

**Summary of CXE Kit™ Advantages:**

The **Extended CSE Dilution KIT** contains CSE, a vial of endotoxin stabilizing solution, a pack of depyrogenated dilution tubes and a CSE and LAL lot-specific Certificate of Analysis. The kit allows long-term use and storage of a standard dilution series in a way that is fully regulatory compliant. On a cumulative basis, the kit saves much time and effort because of the long shelf life of the endotoxin solutions. The kit reduces the uncertainty and inconvenience associated with daily use of a SDS prepared by other methods.

**NEW ENDOSAFE PAGER NUMBER FOR**
**EMERGENCY TECHNICAL HELP: 843-965-0897**

## Charles River Endosafe and Y2K

As the new millennium approaches, concerns over Y2K issues continue to grow. Charles River Endosafe, in keeping with our commitment to superior customer service and support, provides the following statement regarding the ELX808™ incubating microplate reader and Biolise™ software.

*Biotek, Inc.,* the manufacturer of the ELX808™ microplate reader, has provided a statement of compliance for the ELX808™ incubating microplate reader which may be obtained from **www.Biotek.com**.

A detailed testing matrix and the results for the Y2K testing of the Biolise™ software are available on the Endosafe website **www.endosafe.com**. *These results have been provided by Biolise CDL*. A copy may also be obtained by submitting a written request to Charles River Laboratories, Attention year 2000 Coordinator, 251 Ballardvale Street, Wilmington, MA 01887.

Charles River Endosafe makes every effort to ensure the products we sell are Y2K compliant. However, Charles River Endosafe offers no guarantees either expressed or implied that the products it sells are Y2K compliant. *Charles River has not and does not verify the contents of the year 2000 compliance statements provided by Biolise CDL.* It is essential that the user evaluate the platform used in conjunction with the Biolise™ software to ensure that the system is Y2K compliant. Any action or non-action taken by a party based on Year 2000 information provided by Charles River Endosafe is solely at the Party's risk.

**NOTICE IS HEREBY GIVEN that the Year 2000 Statement set forth herein is being designated as a Year 2000 Readiness Disclosure in accordance with the Year 2000 Information and Readiness Disclosure Act.**

## CALENDAR

March 1-5 - PDA Spring Conference, Orlando

Presentation by Dr. Cooper: "Assigning Endotoxin Limits to New Chemical Entities".

March 11-12, 1999 – NEW DEPYROGENATION COURSE OFFERED BY PDA: The PDA is offering a new course for validating depyrogenation cycles by LAL techniques. Participants in this laboratory-intensive course will learn how to prepare and assay endotoxin indicators, quantify endotoxin reduction by gel-clot and kinetic methods, and use thermoprobe systems to assess heat distribution and penetration values within an oven. The course is offered at the new Baltimore training facility and will be taught by Dr. James Cooper and Dr. Mike Korczynsky, PDA-TRI Director. Contact PDA, 301-986-0293, ext. 131, or e-mail: www.pda-tri.org.

Annual LAL Workshop - Charleston, SC (See registration form in this newsletter.)

Gel-Clot - June 23-26, 1999; Quantitative - June 28-July 1

For more information contact Frances Cooper: phone 843-795-7316; fax: 843-795-7221

**CHARLESTON LAL WORKSHOPS:**

LAL users have been trained in Dr. Cooper's Charleston Workshop since 1978. The unique feature of this workshop is a combination of classroom and lab sessions where the lecture series is reinforced by supervised lab experience. The Workshop is designed to qualify analysts for applying LAL test methods to pharmaceuticals and medical devices using the latest technology.

The **GEL-CLOT** course offers basic information including the properties of LAL-RM,

## LAL WORKSHOP

## REGISTRATION FORM

**Name**

native and standard endotoxins, sources and solutions for LAL-Test interference, regulatory issues and an introduction to kinetic LAL methods. Of interest to supervisory personnel are the discussions on setting in-process endotoxin limits, acceptance criteria and other critical QA issues.

The **QUANTITATIVE** course is designed for those with knowledge of basic LAL techniques who wish to gain knowledge of kinetic systems for measuring endotoxins. Lectures are supplemented with case studies and supervised lab exercises presenting new-generation equipment, software and reagents. Discussions include a review of kinetic methods, regulatory issues, resolution of interference unique to kinetic LAL, strategies for in-process control, trend analysis with new database software and qualification of automated systems. Ways to select equipment and initiate kinetic LAL programs are emphasized.

**WORKSHOP SITE AND ACCOMMODATIONS**: The Workshop will be held at the HAWTHORN SUITES HOTEL, 181 Church Street. Hawthorn Suites Hotel is located in Charleston's historic City Market area and is within walking distance to restaurants, Waterfront Park, historic homes and shopping. Valet parking is available at a daily rate. All major rental car agencies serve the Charleston International Airport. Limousine service is available from the airport to the hotel.

**HOTEL ACCOMMODATIONS**: **A BLOCK OF ROOMS WILL BE HELD UNTIL MAY 23, 1999**, for workshop participants at a rate of $99

**Position**

_____

**Company/Organization**

_____

**Street Address**

_____

**City/State/Zip**

_____

**Phone & Fax**

**LAL Experience: _____Years**

## REGISTRATION

**_____GEL CLOT, June 23-26, 1999: ($500)**

**_____QUANTITATIVE, June 28-July 1: ($550)**

**_____BOTH COURSES, gel & quant: ($900)**

**MAKE CHECK PAYABLE TO:**

**ENDOSAFE LAL WORKSHOP**

**MAIL TO:**

**FRANCES COOPER**

for single or double occupancy. This rate includes breakfast; rates are subject to state and local taxes. After May 23, reservations will be accepted on a space available basis only. Please make reservations early and identify yourself as an Endosafe LAL Workshop participant. Please call the hotel to reserve your room. **HAWTHORN SUITES HOTEL: 843-577-2644**

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**FOR ADDITIONAL INFORMATION:**

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