LAL UPDATE®

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Dear LAL User:

This issue of the LAL UPDATE will examine how Control Standard Endotoxin (CSE) formulation, i.e., non-endotoxin excipients in the preparation, affects recovery in inhibition/enhancement tests. The effect of non-endotoxin excipients in depyrogenation has already been covered in detail in LAL UPDATE Vol. 8, No. 1. This issue of the LAL UPDATE will conclude with our regular GLUCAN CORNER and CALENDAR.

I would also like to take this opportunity to introduce the newest member of Associates of Cape Cod's management team, Dr. Malcolm A. Finkelman, who will head our Product Development department. Dr. Finkelman comes to us from Genex Corporation where he was Director of Process Development. He earned his Ph.D. in microbiology and immunology from the University of Western Ontario, Canada.

Sincerely,

Thomas J. Novitsky, Ph.D.
Editor

Factors Affecting the Recovery of Endotoxin

The first reference standard endotoxin (RSE) produced by the USFDA for widespread use was EC-2. The lipopolysaccharide component for this standard was obtained by phenol-water (Westphal) extraction of *Escherichia coli* O113:H10:K- or the "Braude" strain. It was prepared for the FDA's then Bureau of Biologics by Dr. Jon A. "Tony" Rudbach. The chemistry and biological activity of the preparation has been thoroughly described in the literature (1, 2, 3). At the recommendation of Dr. Rudbach, the endotoxin was prepared with serum albumin as a filler. The albumin served as a cryoprotectant during freeze-drying, as a solubilizing agent, and as a visual guide (the 0.5 µg of endotoxin per vial was invisible by itself). Associates of Cape Cod, Inc. obtained from Dr. Rudbach most of the endotoxin that was left over from the FDA's order. This amounted to several grams, so Associates was able to provide control standard endotoxin (CSE) from the same bulk preparation as EC-2 to its customers for many years.*

The early lots of Associates' CSE also contained human serum albumin (2.5 mg per vial or 2.5 mg albumin per 1 µg endotoxin) as a filler. As LAL users became more sophisticated and began to refine the inhibition/enhancement assay, they soon discovered that albumin could affect the recovery of endotoxin from samples. The first product where this effect was noted was common physiological saline. When we added our CSE to 0.9% NaCl solution, only 1/2 of the added amount could be recovered. (Note: Although this is within the statistical limits of the test, it was extremely consistent.) We then tried the RSE (EC-2) with similar results (Table 1). With endotoxin prepared

without albumin (or any other filler), 100% recovery was obtained. We also tested the recovery of a highly purified (electrodialysed) endotoxin, Novo-Pyrexal, with similar success (Table 2). ACC immediately began working on a filler-free CSE product and in 1979 marketed our first lot (lot #6) without albumin. The FDA was also notified of our findings regarding albumin fillers and since EC-2 was running out, a filler-less replacement was proposed and tested. Unfortunately lots EC-3 and EC-4 did not work as expected (a filler makes it easier to handle liquid endotoxin and prevents adsorption to filling lines, etc.). The FDA then turned to Don Mills at Mallinckrodt, Inc., who had experience with non-albumin fillers. Ultimately EC-5 was produced by Mallinckrodt for combined use by the FDA and the USP. To date, EC-5 has not shown the recovery problems typical of the albumin-containing standards.** It should be noted however, that because the RSE is expensive (current USP price is $90.00) most people do not use it for inhibition/enhancement recovery. Since EC-5 contains 1000 times more lactose and 100 times more PEG (by weight) than endotoxin, it is still possible that recovery problems may arise, especially with the quantitative methods which don't have the twofold cushion of the gel-clot test.

Since the problem associated with albumin as a filler is general knowledge among those experienced with the LAL test, it came as quite a surprise to this editor to find a new CSE standard (*E. coli* O111:B4) containing albumin currently included in a European multicenter study (QC-ET)! In fact, there are two standards included in the study, varying only by the concentration of albumin (1.3 mg and 1.4 mg/vial). A description of a similar standard (1.25 mg O111:B4/vial) has appeared in the literature, apparently to give some credibility to a CSE which would need no further comparison to a primary standard by a user (4). Our preliminary tests of these preparations in water and saline gave the expected difference in potency (Table 3). It will be interesting to expand these tests to see if a statistical difference exists.

It is unfortunate that the QC-ET study and others compare only water standard curves and single lots of standard. Used in this manner almost any endotoxin would pass muster as a CSE. However, CSE's are used for recovery studies or as product positive controls. In addition, they are also used for depyrogenation studies. I wish the endotoxin standard makers of the world make note of this. Associates of Cape Cod is currently investigating a new formulation of CSE (with filler) which will have all the desirable properties required for a standard endotoxin, i.e., high stability, excellent recovery, ease of reconstitution, etc. Until such a standard is available, it is extremely important that LAL users beware - **ENDOTOXINS THAT APPEAR SIMILAR IN POTENCY WHEN TESTED IN WATER MAY NOT SHOW SIMILAR POTENCY IN PRODUCT DUE TO THE PRESENCE OF CERTAIN FILLERS.**

*ACC's original stock of endotoxin will run out this year. A replacement stock prepared by Dr. Rudbach (now at Ribi Immunochem) from the same "Braude" strain, is currently being tested by ACC.

**Note, lactose/PEG fillers do affect depyrogenation. It is wise not to use any type of filler-containing endotoxin for depyrogenation validations. (See LAL Update Vol. 8, No.1.)

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TABLE 1. Potency of EC-2 in water and saline with gel-clot test

LAL Lot	End Point ¤	
	Water	Saline
USFDA Ref. #8	0.03	0.06
ACC lot # 52-76 -245	0.008	0.03

¤ng/ml of EC-2

TABLE 2. Potency of *Salmonella abortus equi* endotoxin(Novo-Pyrexal) in water and saline with gel-clot test

LAL Lot	End Point ¤¤	
	Water	Saline
ACC lot # 52-74-243	0.025	0.025
ACC lot # 52-75-244	0.05	0.05
ACC lot # 52-76-245	0.013	0.013

¤¤ng NP/ml

TABLE 3. Preliminary comparison of CSE Standards in water and saline ❖

CSE	Dilution					
	1:2	1:4	1:8	1:16	1:32	1:64
Water						
QC/ET *	+	+	+	+	+	-
ACC**	+	+	+	+	-	-
Saline						
QC/ET	+	+	+	+	-	-
ACC	+	+	+	+	-	-

* (1.3 mg albumin/vial)
** lot 52 (no albumin)
❖ Pyrotell lot # 99-78-421

Glucan Corner

References to recent work on β-(1,3)-glucans include many concerning structural changes which can be related to physiological functions and some concerning their ability to lower blood cholesterol. There are a number of references on the isolation of β-(1,3)-glucan binding proteins and β-(1,3) receptors and others relating to the effect of glucans on the reticuloendothelial system and their role in immunopotentiation.

One paper discusses the role of β-(1,3)-glucans in the formation of granulomas in liver cells. Others describe the binding of β-(1,3) -glucans to fibrinogen, activation of Factor XII and enhancement of clot formation in human plasma.

The response of the *Limulus* amebocyte lysate test to β-(1,3)-glucans has been addressed by Roslansky and Novitsky, Tanaka et al., Kambayashi et al., and Tsuchiya et al.

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Calendar

92nd American Society for Microbiology
New Orleans, LA May 26-30,1992
Visit Associates of Cape Cod, Inc. at Booth 1131

Medical Design and Manufacturing East 92
June 2-4, 1992
Jacob H. Javits Convention Center
New York City
"Medical Devices: Special issues concerning bacterial endotoxin contamination and detection."
by Marilyn J. Gould, Ph.D.
(In Cleanrooms and Controlled Environments Forum-Session 207, June 2, 1992)

American Veterinary Medical Association
129th Annual Meeting
August 1-5, 1992 Boston, MA
"Biomedical Applications of *Limulus*: The Horseshoe Crab"
by Thomas J. Novitsky, Ph.D.

Second Conference of the International Endotoxin Society
Penta Hotel, Vienna, Austria
August 17-20, 1992