LAL UPDATE®

Volume 10, No. 2 **June 1992**

Dear LAL User,

This issue of the UPDATE introduces a new product, END-X ™B15 beads. This product removes endotoxin like our earlier product, the END-X filter, but using a different sample handling format. Although this product is directed mainly at research and development applications, QC/QA departments may find END-X B15 especially suited for "cleaning-up" small samples of new products prior to LAL validation. Occasionally, a new product has a residual pyroburden or "background" endotoxin, not sufficient to fail the product, but just enough to make validation of the product difficult if not impossible. With simple solutions, ultrafiltration has been used successfully to reduce the endogenous endotoxin to allow successful validation. However, ultrafiltration will not work with most biologics. END-X products were specifically designed to work in protein solutions. Since the BET allows pretreatment of sample to facilitate LAL validation. It may now be possible to validate new and existing products containing low but detectable amounts of endotoxin.

This issue concludes with our regular USP Says and CALENDAR features.

Sincerely,

Thomas J. Novitsky, Ph.D.
Editor

END-X™ B15: New Endotoxin Removal Device Added to Product Line

by Malcolm A. Finkelman, Ph.D.

Associates of Cape Cod, Inc. is pleased to announce the introduction of the END-X B15 endotoxin removal device. The unit consists of a microcentrifuge tube containing an affinity resin composed of endotoxin neutralizing protein (ENP) immobilized onto glass microspheres. The immobilized ENP provides high affinity binding of endotoxin to the solid phase. The high density of the microspheres provides for very simple and rapid separation from the liquid phase. The combination of high specificity and capacity, coupled with the extreme simplicity of the system, promises to greatly aid researchers in eliminating endotoxin contamination from valuable research reagents.

For years, ACC has advanced the methodology and reagents for the detection and quantitation of endotoxin. Recently, we have focused an increasing amount of attention on removal of endotoxin in contaminated solutions. Our customers saw the first results of this program with the launch of the END-X 5, a microfiltration device with ENP covalently coupled to the membrane. Feedback from users suggested that there was a need for an even simpler approach to small sample clean-up.

Accordingly, ACC has developed the END-X B (bead) line. The first of this family of products is the B15, which is aimed at the clean-up of small volumes of valuable samples.

This product is being offered as a kit which contains two sets of endotoxin-free tubes. The first set contains the immobilized ENP resin, and is used to extract the endotoxin from the liquid phase. The second set are receiver tubes. It is critical that the newly decontaminated solution be stored in an endotoxin-free container. ACC has decided to supply these to the user in order to ensure freedom from recontamination.

END-X B15 Performance

Our testing has shown that the END-X B15 is extremely effective at removing endotoxin from contaminated solutions. Typically, a greater than 99% reduction is observed even where the solution endotoxin burden is significantly greater than the recommended level of 50 nanograms of endotoxin. The data in the accompanying advertisement shows this to be true for a broad range of solutions. In addition, the non-specific binding of proteins in the treated solutions is quite low. Figure 1. illustrates the minimal loss of protein over time. Although our data suggests generally low non-specific binding, users should try to avoid working with minute amounts of protein.

ENP: Another valuable protein from the Horseshoe Crab

Users of LAL reagent have long been accustomed to the benefits of the extract from the amebocytes of the horseshoe crab (*Limulus polyphemus*). You should be aware that ENP, the active ingredient of the END-X product line, is also extracted from the *Limulus* amebocytes. Thus, the animal not only provides the most sophisticated means of detection of endotoxin, but also the most effective way of removing it from solution.

Process Applications

Additional END-X series products have been requested for process applications to remove endotoxin from valuable pharmaceutical products. ACC is currently developing additional devices for these applications. We will be launching two more products this summer, which will allow multi-liter batches to be effectively decontaminated using Luer-ended cartridges containing immobilized ENP-resin. In support of process applications, ACC will offer complete documentation of these devices and their components. ACC will also provide ELISA methodology to allow customers to validate low protein leaching from the resin.

New Applications

The Product Development group at ACC is very interested to hear from you with respect to your application needs and your suggestions for improvements to our current devices. If you wish to discuss any aspect of the End-X program, please call. We look forward to hearing from you.

Figure 1. END-X B15 Non-Specific Protein Binding

Sample	Exposure Time and % Remaining		
	0 Hours	8 Hours	24 Hours
rDNA Enzyme	100%	100%	96.6%
	0 Hours	2 Hours	6 Hours
Fetal Bovine Serum	100%	105.3%	96.4%

References

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2. Wainwright, N. R., R. J. Miller, E. Paus, T. J. Novitsky, M. A. Fletcher, T. M. McKenna, and T. Williams. 1990. Endotoxin binding and neutralizing activity by a protein from *Limulus polyphemus*. p. 315-325. In A. Nowotny, J. J. Spitzer,and E. J. Ziegler, (ed) Cellular and molecular aspects of endotoxin reactions. Elsevier Science Publishers B.V., Amsterdam.

3. Warren, H. S., M. L. Glennon, N. Wainwright, S. F. Amato, K. M. Black, S. J. Kirsch, G. R. Riveau, R. I. Whyte, W. M. Zapol and T. J. Novitsky. 1992. Binding and neutralization of endotoxin by *Limulus* antilipopolysaccharide factor. Infection and Immunity. **60**:2506-2513.

USP Changes to *Bacterial Endotoxin Test* from *Pyrogen Test* for Articles in the 6th Supplement to USP XXII

Because of the large number of changes from the *Pyrogen Test* to the *Bacterial Endotoxin Test* in the 6th Supplement to USP XXII, the changes are summarized in tablular form. Only the first article is given in its entirety. Articles are grouped according to whether the endotoxin limit is expressed in EU/mg, EU/mL, EU/µg or EU/USP unit.

"Sterile Acetazolamide Sodium — Bacterial endotoxins — When tested as directed under *Bacterial Endotoxins Test* <85>, it contains not more than 0.5 USP Endotoxin Unit per mg of acetazolamide."

Article	Limit (USP Endotoxin Unit(s) per mg or µg)
Sterile Acetazolamide Sodium	0.5
Amikacin Sulfate Injection	0.33
Aminohippurate Sodium Injection	0.04
Sterile Amobarbital Sodium	0.4
Sterile Ampicillin Sodium	0.15
Anileridine Injection	7.2
Ascorbic Acid Injection	1.2
Atropine Sulfate Injection	55.6
Azathioprine Sodium for Injection	1.0
Benztropine Mesylate Injection	55.6
Sterile Betamethasone Sodium Phosphate and Betamethasone Acetate Suspension	29.2
Bethanechol Chloride Injection	25.0
Biperiden Lactate Injection	83.3
Bupivacaine in Dextrose Injection	1.8
Bupivacaine and Epinephrine Injection	1.6
Bupivacaine Hydrochloride Injection	2.5
Butorphanol Tartrate Injection	88.0
Caffeine and Sodium Benzoate Injection	0.7
Calcium Chloride Injection	0.2
Carboprost Tromethamine Injection	714.3
Sterile Chlordiazepoxide Hydrochloride	3.57
Chloroquine Hydrochloride Injection	0.7
Chlorothiazide Sodium for Injection	0.3
Chlorpheniramine Maleate Injection	8.8
Chlorpromazine Hydrochloride Injection	6.9
Chlorprothixene Injection	6.9
Sterile Cilastatin Sodium	0.23
Codeine Phosphate Injection	5.8
Colchicine Injection	166.7
Cyanocobalamin Injection	0.4 /µg
Cyclophosphamide for Injection	0.20
Cysteine Hydrochloride Injection	0.7
Sterile Cytarabine	0.07
Dactinomycin	100
Dactinomycin for Injection	100.0
Desoxycorticosterone Acetate Injection	71.4
Sterile Desoxycorticosterone Pivalate Suspension	2.78
Sterile Dexamethasone Acetate Suspension	21.7
Dexamethasone Sodium Phosphate Injection	31.3
Dibucaine Hydrochloride Injection	35.7
Dicyclomine Hydrochloride Injection	17.2
Diethylstilbestrol Injection	0.7
Diethylstilbestrol Diphosphate Injection	0.7
Digitoxin Injection	111.0
Digoxin Injection	200.0
Diphenhydramine Hydrochloride Injection	3.4
Doxapram Hydrochloride Injection	3.3
Doxorubicin Hydrochloride Injection	2.2
Doxycycline Hyclate for Injection	1.14
Emetine Hydrochloride Injection	5.4
Ephedrine Sulfate Injection	1.7
Epinephrine Injection	357.0
Ergotamine Tartrate Injection	357.0
Sterile Estradiol Suspension	250.0
Sterile Estrone Suspension	88.0
Ethacrynate Sodium for Injection	5.0
Ethylnorepinephrine Hydrochloride Injection	172.4
Folic Acid Injection	357.1
Gallamine Triethiodide Injection	5.0
Glycopyrrolate Injection	555.5
Haloperidol Injection	71.4
Histamine Phosphate Injection	125.0
Hydralazine Hydrochloride Injection	1.45
Sterile Hydrocortisone Suspension	1.25
Hydrocortisone Sodium Phosphate Injection	1.25
Hydrocortisone Sodium Succinate for Injection	1.25
Hydromorphone Hydrochloride Injection	88.0
Hydroxocobalamin Injection	0.4
Sterile Hydroxystilbamidine Isethionate	1.1
Hydroxyzine Hydrochloride Injection	3.6
Hyoscyamine Sulfate Injection	714.3
Sterile Imipenem	0.23
Imipramine Hydrochloride Injection	5.0
Sterile Indocyanine Green	7.1
Iophendylate Injection	0.9
Isoniazid Injection	0.3
Ketamine Hydrochloride Injection	0.4
Labetalol Hydrochloride Injection	1.2
Levorphanol Tartrate Injection	125.0
Lidocaine and Epinephrine Injection	0.7
Lidocaine Hydrochloride Injection	1.1
Sterile Lidocaine Hydrochloride	1.1
Lidocaine Hydrochloride and Dextrose Injection	1.1

Lincomycin Hydrochloride	0.5
Lincomycin Hydrochloride Injection	0.5
Magnesium Sulfate Injection	0.09
Mechlorethamine Hydrochloride for Injection	12.5
Menadiol Sodium Diphosphate Injection	25.0
Menadione Injection	58.3
Meperidine Hydrochloride Injection	2.4
Mephentermine Sulfate Injection	7.8
Mepivacaine Hydrochloride Injection	0.8
Mepivacaine Hydrochloride and Levonordefrin Injection	0.8
Meprylcaine Hydrochloride and Epinephrine Injection	0.8
Mesoridazine Besylate Injection	7.0
Metaraminol Bitartrate Injection	3.5
Methadone Hydrochloride Injection	8.8
Methocarbamol Injection	0.2
Methohexital Sodium for Injection	0.5
Methotrimeprazine Injection	17.9
Methoxamine Hydrochloride Injection	20.0
Methyldopate Hydrochloride Injection	0.5
Methylergonovine Maleate Injection	1.7/ µg
Methylprednisolone Sodium Succinate for Injection	0.17
Metoclopramide Injection	2.5
Metocurine Iodide Injection	12.5
Metoprolol Tartrate Injection	25.0
Morrhuate Sodium Injection	1.4
Nalorphine Hydrochloride Injection	11.6
Niacin Injection	3.5
Niacinamide Injection	3.5
Norepinephrine Bitartrate Injection	83.4
Orphenadrine Citrate Injection	5.8
Oxymorphone Hydrochloride Injection	238.1
Papaverine Hydrochloride Injection	2.9
Pentazocine Lactate Injection	5.8
Pentobarbital Sodium Injection	0.8
Perphenazine Injection	35.7
Phenobarbital Sodium Injection	0.3
Sterile Phenobarbital Sodium	0.8
Phentolamine Mesylate for Injection	5.8
Phenylephrine Hydrochloride Injection	25.0
Phenytoin Sodium Injection	0.3
Potassium Phosphates Injection	1.10
Prednisolone Sodium Phosphate Injection	5.0
Prednisolone Sodium Succinate for Injection	5.8
Sterile Prednisolone Tebutate Suspension	8.8
Prilocaine Hydrochloride Injection	0.9
Prilocaine and Epinephrine Injection	0.9
Procaine Hydrochloride Injection	0.6
Sterile Procaine Hydrochloride	0.6
Procaine Hydrochloride and Epinephrine Injection	0.6
Procaine and Phenylephrine Hydrochlorides Injection	0.6
Procaine and Tetracaine Hydrochlorides and Levonordefrin Injection	0.6
Prochlorperazine Edisylate Injection	17.9
Promazine Hydrochloride Injection	1.8
Promethazine Hydrochloride Injection	5.0
Sterile Propantheline Bromide	11.6
Propiomazine Hydrochloride Injection	4.6
Propoxycaine and Procaine Hydrochlorides and Levonordefrin Injection	0.8
Propoxycaine and Procaine Hydrochlorides and Norepinephrine Bitartrate Injection	0.8
Propranolol Hydrochloride Injection	55.6
Protamine Sulfate Injection	7.04
Pyridoxine Hydrochloride Injection	0.4
Quinidine Gluconate Injection	0.6
Reserpine Injection	71.5
Riboflavin Injection	7.1
Rifampin for Injection *	0.2
Scopolamine Hydrobromide Injection	555.0
Secobarbital Sodium Injection	0.9
Sterile Secobarbital Sodium	0.9
Sodium Thiosulfate Injection	0.03
Succinylcholine Chloride Injection	2.0
Sterile Succinylcholine Chloride	2.0
Sulfadiazine Sodium Injection	0.1
Terbutaline Sulfate Injection	1250.0
Sterile Testolactone Suspension	3.5
Sterile Testosterone Suspension	3.5
Tetracaine Hydrochloride Injection	0.7
Sterile Tetracaine Hydrochloride	0.7
Tetracaine Hydrochloride in Dextrose Injection	1.0
Thiamine Hydrochloride Injection	3.5
Thiamylal Sodium for Injection	1.0
Thiethylperazine Malate Injection	35.8
Thiopental Sodium for Injection	1.0
Thiothixene Hydrochloride Injection	88.0
Thiothixene Hydrochloride for Injection	88.0
Tobramycin Sulfate	2.00
Tobramycin Sulfate Injection	2.00
Sterile Tobramycin Sulfate	2.00
Tolazoline Hydrochloride Injection	0.8
Sterile Tolbutamide Sodium	0.35
Sterile Triamcinolone Acetonide Suspension	4.4
Sterile Triamcinolone Diacetate Suspenison	7.1
Sterile Triamcinolone Hexacetonide Suspension	17.2
Trifluoperazine Hydrochloride Injection	172.0
Triflupromazine Hydrochloride Injection	5.8
Tubocurarine Chloride Injection	10.0
Verapamil Injection	16.7
Sterile Vidarabine	0.5
Sterile Vinblastine Sulfate	10.0
Warfarin Sodium for Injection	24.0

* Note special procedure; dissolve in methanol, then dilute in Tris buffer, etc. See page 2898 in the 6th Supplement to USP XXII.

Article	Limit (USP Endotoxin Unit(s) per mL)
Alcohol in Dextrose Injection	0.5
Methylene Blue Injection	2.5
Potassium Chloride in Lactated Ringer's and Dextrose Injection	0.5
Ringer's Injection	0.5
Lactated Ringer's Injection	0.5

Article	Limit (USP Endotoxin Unit(s) perUnit)
Corticotropin Injection [1]	3.1
Corticotropin for Injection [1]	3.1
Repository Corticotropin Injection [1]	3.1
Sterile Corticotropin Zinc Hydroxide Suspension [1]	3.1
Chorionic Gonadotropin for Injection [2]	0.03
Menotropins [3]	2.5
Menotropins for Injection [3]	2.5
Sterile Penicillin G Procaine [4]	0.01
Sterile Penicillin G Procaine with Aluminum Stearate Suspension [4]	0.01
Posterior Pituitary Injection [5]	17.0

[1] USP Endotoxin Units per USP Cortocotropin Unit
[2] USP Endotoxin Unit per USP Chorionic Gonadotropin Unit
[3] USP Endotoxin Units per USP Follicle-stimulating Hormone Unit
[4] USP Endotoxin Unit per 100 Penicillin G Units
[5] USP Endotoxin Unit per Posterior Pituitary Units

Clindamycin Phosphate, USP XXII page 323 and page 2598 of the Fifth Supplement. The following changes place the *Bacterial endotoxin* test under *Other requirements* to specify that Clindamycin Phosphate intended for making Clindamycin Phosphate Injection must comply with the requirements of the *Bacterial endotoxins* test (as specified under *Sterile Clindamycin Phosphate*).

Lincomycin Hydrochloride, USP XXII page 771, page 2637 of the Fifth Supplement, and page 2334 of PF 17(5), [Sept.-Oct. 1991]. The following changes place the *Bacterial endotoxins* test under *Other requirements* to specify that Lincomycin Hydrochloride intended for making Lincomycin Hydrochloride Injection must comply with the requirements of the *Bacterial endotoxins* test (as specified under Sterile Lincomycin Hydrochloride).

The following proposed revisions concerning the **Pyrogen Test** <151> are included in the 6th Supplement to USP XXII.

1. Cefotiam for Injection. **Pyrogen** — It meets the requirements of the *Pyrogen Test* <151>, the test dose being 1.0 mL per kg of a solution prepared by diluting Cefotiam for Injection with Sterile Water for Injection to a concentration of 40 mg of cefotiam per mL.
2. Sterile Cefotiam Hydrochloride. **Pyrogen** — It meets the requirements of the *Pyrogen Test*<151>, the test dose being 1.0 mL per kg of a solution in pyrogen-free sodium carbonate solution (prepared by dissolving 25.6 g of sodium carbonate, previously heated at 170° for not less than 4 hours, in 1000 mL of Sterile Water for Injection) containing 40 mg per mL.

The following proposed new monograph is included in the Pharmacopeial Previews section of Pharmacopeial Forum, Vol. 18, No. 1, Jan.-Feb. 1992.

Article	Limit
Sodium Fluoride F 18 Injection	To come

The following proposed changes to monographs are included in the In-Process Revisions Section of the Pharmacopeial Forum, Vol. 18, No. 1, Jan.-Feb. 1992.

Article	Limit
Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation	2.80 mL
Dihydroergotamine Mesylate Injection	357.0 mg
Dobutamine for Injection Concentrate	5.56 mg
Iohexol Injection	0.2 mg
Water O 15 Injection	175/V per mL

<1045> Biotechnology-derived Articles (see page 2895)

The following proposed changes to monographs are included in the In-Process Revisions Section of the Pharmacopeial Forum, Vol. 18, No. 2 , Mar.-Apr. 1992.

Article	Limit
Aminocaproic Acid Injection	0.05 mg
Ammonium Chloride Injection	1.72 mEq
Benzylpenicilloyl Polylysine Injection	1250.0 mL
Cephalothin Sodium	0.13 mg
Cephalothin Sodium Injection	0.13 mg
Cephalothin Sodium for Injection	0.13 mg
Sterile Cephalothin Sodium	0.13 mg
Cisplatin for Injection	2.0 mg
Dacarbazine for Injection	0.52 mg
Daunorubicin Hydrochloride for Injection	4.3 mg
Dextrose Injection	10.0 mL
Dextrose and Sodium Chloride Injection	10.0 g
Diatrizoate Meglumine Injection	1.1 mL
Diatrizoate Meglumine Injection and Diatrizoate Sodium Injection	1.8 mL
Diatrizoate Sodium Injection	5.6 mL
Dihydroergotamine Mesylate Injection	175.0 mg
Dobutamine Hydrochloride for Injection	2.08 mg
Edetate Disodium Injection	0.2 mg
Ergonovine Maleate Injection	700.0 mg
Fluorouracil Injection	0.33 mg
Sterile Gentamicin Sulfate	1.7 mg
Mannitol Injection	0.04 g
Mannitol in Sodium Chloride Injection	0.04 g
Potassium Chloride in Dextrose Injection	8.8 mEq
Potassium Chloride in Dextrose and Sodium Chloride Injection	8.8 mEq
Potassium Chloride in Sodium Chloride Injection	8.8 mEq
Protamine Sulfate Injection	7.0 mg
Sterile Sodium Nitroprusside	3.67/μg
Vincristine Sulfate for Injection	100.0 mg

Calendar

2nd Annual Meeting on Advances in Prevention
and Treatment of Endotoxemia & Sepsis
POSTER- "Endotoxin Clean-up by Immobilized Endotoxin
Neutralizing Protein from *Limulus* Polyphemus"
by T. J. Novitsky, Ph.D., S. B. Boyd, and M.A. Finkelman, Ph.D.
Philadelphia, PA June 21-23, 1992

"Rationale in Choosing an LAL Method"
by Thomas J. Novitsky, Ph.D.
Ministerio de Sanidad
Madrid, Spain July 7, 1992

American Society for Quality Control Biomedical Focus VI
July 27-29, 1992 Bloomington, MN
"LAL: Beyond the Basics"
by Marilyn J. Gould, Ph.D.

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

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P.O. Box 224 · Woods Hole, Massachusetts 02543-0224
Tel (508) 540-3444 · Fax (508) 540-8680