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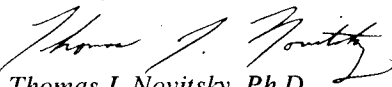
June 1992

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 contami-

nated 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.

References

1. Alpert, G., G. Baldwin, C. Thompson, N. Wainwright, T. J. Novitsky, Z. Gillis, G. R. Siber, J. Parsonnet and G. R. Fleisher. 1992. *Limulus* antilipoplysaccharide factor protects rabbits from meningococcal endotoxin shock. *J. Infect. Dis.* 165:494-500.
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* antilipoplysaccharide factor. *Infection and Immunity.* 60:2506-2513.

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%
Fetal Bovine Serum	100%	105.3%	96.4%

END-X™ B15

Product ordering information

To Order: Call
800-LAL-TEST

Technical help: Call
800-848-3248

Product Code: END-X B15

Description: END-X B15 Etox
Affinity Resin

Expiration Date: 18 months
from date of preparation.

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 tabular 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/ μ g 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 μ g)		
Sterile Acetazolamide Sodium	0.5	Dactinomycin	100
Amikacin Sulfate Injection	0.33	Dactinomycin for Injection	100.0
Aminohippurate Sodium Injection	0.04	Desoxycorticosterone Acetate Injection	71.4
Sterile Amobarbital Sodium	0.4	Sterile Desoxycorticosterone Pivalate Suspension	2.78
Sterile Ampicillin Sodium	0.15	Sterile Dexamethasone Acetate Suspension	21.7
Anileridine Injection	7.2	Dexamethasone Sodium Phosphate Injection	31.3
Ascorbic Acid Injection	1.2	Dibucaine Hydrochloride Injection	35.7
Atropine Sulfate Injection	55.6	Dicyclomine Hydrochloride Injection	17.2
Azathioprine Sodium for Injection	1.0	Diethylstilbestrol Injection	0.7
Benztrapine Mesylate Injection	55.6	Diethylstilbestrol Diphosphate Injection	0.7
Sterile Betamethasone Sodium Phosphate and Betamethasone Acetate Suspension	29.2	Digitoxin Injection	111.0
Bethanechol Chloride Injection	25.0	Digoxin Injection	200.0
Biperiden Lactate Injection	83.3	Diphenhydramine Hydrochloride Injection	3.4
Bupivacaine in Dextrose Injection	1.8	Doxapram Hydrochloride Injection	3.3
Bupivacaine and Epinephrine Injection	1.6	Doxorubicin Hydrochloride Injection	2.2
Bupivacaine Hydrochloride Injection	2.5	Doxycycline Hyclate for Injection	1.14
Butorphanol Tartrate Injection	88.0	Emetine Hydrochloride Injection	5.4
Caffeine and Sodium Benzoate Injection	0.7	Ephedrine Sulfate Injection	1.7
Calcium Chloride Injection	0.2	Epinephrine Injection	357.0
Carboprost Tromethamine Injection	714.3	Ergotamine Tartrate Injection	357.0
Sterile Chlordiazepoxide Hydrochloride	3.57	Sterile Estradiol Suspension	250.0
Chloroquine Hydrochloride Injection	0.7	Sterile Estrone Suspension	88.0
Chlorothiazide Sodium for Injection	0.3	Ethacrynate Sodium for Injection	5.0
Chlorpheniramine Maleate Injection	8.8	Ethylnorepinephrine Hydrochloride Injection	172.4
Chlorpromazine Hydrochloride Injection	6.9	Folic Acid Injection	357.1
Chlorprothixene Injection	6.9	Gallamine Triethiodide Injection	5.0
Sterile Cilastatin Sodium	0.23	Glycopyrrolate Injection	555.5
Codeine Phosphate Injection	5.8	Haloperidol Injection	71.4
Colchicine Injection	166.7	Histamine Phosphate Injection	125.0
Cyanocobalamin Injection	0.4 / μ g	Hydralazine Hydrochloride Injection	1.45
Cyclophosphamide for Injection	0.20	Sterile Hydrocortisone Suspension	1.25
Cysteine Hydrochloride Injection	0.7	Hydrocortisone Sodium Phosphate Injection	1.25
Sterile Cytarabine	0.07	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
		Iopendylate 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	Promazine Hydrochloride Injection	1.8
Lincomycin Hydrochloride Injection	0.5	Promethazine Hydrochloride Injection	5.0
Magnesium Sulfate Injection	0.09	Sterile Propantheline Bromide	11.6
Mechlorethamine Hydrochloride for Injection	12.5	Propiomazine Hydrochloride Injection	4.6
Menadiol Sodium Diphosphate Injection	25.0	Propoxycaïne and Procaine Hydrochlorides and Levonordefrin Injection	0.8
Menadione Injection	58.3	Propoxycaïne and Procaine Hydrochlorides and Norepinephrine Bitartrate Injection	0.8
Meperidine Hydrochloride Injection	2.4	Propranolol Hydrochloride Injection	55.6
Mephentermine Sulfate Injection	7.8	Protamine Sulfate Injection	7.04
Mepivacaine Hydrochloride Injection	0.8	Pyridoxine Hydrochloride Injection	0.4
Mepivacaine Hydrochloride and Levonordefrin Injection	0.8	Quinidine Gluconate Injection	0.6
Meprylcaine Hydrochloride and Epinephrine Injection	0.8	Reserpine Injection	71.5
Mesoridazine Besylate Injection	7.0	Riboflavin Injection	7.1
Metaraminol Bitartrate Injection	3.5	Rifampin for Injection *	0.2
Methadone Hydrochloride Injection	8.8	Scopolamine Hydrobromide Injection	555.0
Methocarbamol Injection	0.2	Secobarbital Sodium Injection	0.9
Methohexital Sodium for Injection	0.5	Sterile Secobarbital Sodium	0.9
Methotrimeprazine Injection	17.9	Sodium Thiosulfate Injection	0.03
Methoxamine Hydrochloride Injection	20.0	Succinylcholine Chloride Injection	2.0
Methyldopate Hydrochloride Injection	0.5	Sterile Succinylcholine Chloride	2.0
Methylergonovine Maleate Injection	1.7/ µg	Sulfadiazine Sodium Injection	0.1
Methylprednisolone Sodium Succinate for Injection	0.17	Terbutaline Sulfate Injection	1250.0
Metoclopramide Injection	2.5	Sterile Testolactone Suspension	3.5
Metocurine Iodide Injection	12.5	Sterile Testosterone Suspension	3.5
Metoprolol Tartrate Injection	25.0	Tetracaine Hydrochloride Injection	0.7
Morrhuate Sodium Injection	1.4	Sterile Tetracaine Hydrochloride	0.7
Nalorphine Hydrochloride Injection	11.6	Tetracaine Hydrochloride in Dextrose Injection	1.0
Niacin Injection	3.5	Thiamine Hydrochloride Injection	3.5
Niacinamide Injection	3.5	Thiamylal Sodium for Injection	1.0
Norepinephrine Bitartrate Injection	83.4	Thiethylperazine Malate Injection	35.8
Orphenadrine Citrate Injection	5.8	Thiopental Sodium for Injection	1.0
Oxymorphone Hydrochloride Injection	238.1	Thiothixene Hydrochloride Injection	88.0
Papaverine Hydrochloride Injection	2.9	Thiothixene Hydrochloride for Injection	88.0
Pentazocine Lactate Injection	5.8	Tobramycin Sulfate	2.00
Pentobarbital Sodium Injection	0.8	Tobramycin Sulfate Injection	2.00
Perphenazine Injection	35.7	Sterile Tobramycin Sulfate	2.00
Phenobarbital Sodium Injection	0.3	Tolazoline Hydrochloride Injection	0.8
Sterile Phenobarbital Sodium	0.8	Sterile Tolbutamide Sodium	0.35
Phentolamine Mesylate for Injection	5.8	Sterile Triamcinolone Acetonide Suspension	4.4
Phenylephrine Hydrochloride Injection	25.0	Sterile Triamcinolone Diacetate Suspension	7.1
Phenytoin Sodium Injection	0.3	Sterile Triamcinolone Hexacetonide Suspension	17.2
Potassium Phosphates Injection	1.10	Trifluoperazine Hydrochloride Injection	172.0
Prednisolone Sodium Phosphate Injection	5.0	Triflupromazine Hydrochloride Injection	5.8
Prednisolone Sodium Succinate for Injection	5.8	Tubocurarine Chloride Injection	10.0
Sterile Prednisolone Tebutate Suspension	8.8	Verapamil Injection	16.7
Prilocaine Hydrochloride Injection	0.9	Sterile Vidarabine	0.5
Prilocaine and Epinephrine Injection	0.9	Sterile Vinblastine Sulfate	10.0
Procaine Hydrochloride Injection	0.6	Warfarin Sodium for Injection	24.0
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		

* 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) per Unit)
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 Corticotropin 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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