LAL Update®

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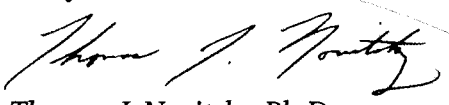
Letter from the President

This issue of the LAL UPDATE begins a two part article which expands on "Inhibition/Enhancement" testing. Since every drug product containing or potentially containing endotoxin differs slightly from the "standard" (i.e. endotoxin in water) with respect to its reactivity with LAL, product-specific validation is necessary to assure us and our regulators that our test result is correct. The LAL attribute of high sensitivity can actually be a drawback if laboratory errors are made, i.e. the introduction of extraneous endotoxin contamination, resulting in an erroneous positive test. On the other hand, many known and unknown chemical substances can lower the reactivity of LAL and result in an erroneous negative test. Even water for injection which meets all the compendial requirements can contain impurities which may influence the LAL assay, hence the requirement for LAL Reagent Water. The rest of the UPDATE contains a few additional items of special interest. From all of us at ACC, have a happy and successful new year!



Thomas J. Novitsky, Ph.D.

Chris Galanos Receives the 2nd Frederik Bang Award

Dr. Chris Galanos of the Max Planck Institute of Immunobiology received the second Frederik Bang Award for an established investigator in the LAL/endotoxin field. Dr. Galanos is a well known endotoxin researcher who has been instrumental in isolating and characterizing lipopolysaccharides.

Dr. Jack Levin, a colleague of Dr. Bang's, received the first Frederik Bang Award at the 1985 International Conference on the Detection of Bacterial Endotoxin with the *Limulus* Amebocyte Lysate Test. The award had been initiated by Dr. Stanley W. Watson to honor the memory of Dr. Bang who described the reactivity of horseshoe crab amebocytes to endotoxin, a discovery which resulted in the LAL test.

Upon the recommendation of Dr. Levin, Associates of Cape Cod, Inc., reinstated the award under the auspices of the International Endotoxin Society. The presentation was made at the 1996 International Endotoxin Society Conference in Japan.

Congratulations Dr. Galanos!

In This Issue...

SPOTLIGHT

Limulus Amebocyte Lysate Regulations Dropped from CFR

In August of 1996, Subpart K, 21 CFR "*Limulus* Amebocyte Lysate" was officially deleted. Although LAL manufacturers and users will not notice any change, the deletion does mark progress of the FDA toward "harmonization." Thus LAL will be treated more like other biological products, relying on information in the product license and amendments rather than specifics in the CFR. Of course, all the Good Manufacturing Practices still apply.

ACC is Inspected by the FDA

Associates of Cape Cod received a surprise inspection by the FDA during October. Fortunately, our recent ISO 9001 certification proved to be a good training exercise and the inspection went smoothly. Credit for our success goes to Dr. Marilyn Gould, Vice President of Regulatory Affairs, Lisa Miller, Quality Assurance Manager, and our environmental monitoring guru, Susan Archambault.

TECHNICAL REPORT

Inhibition or Enhancement Testing: Part 1

By Michael E. Dawson, Ph.D.

Introduction

Inhibition/enhancement testing is carried out to validate an LAL method for testing a specific type of sample. The aim is to demonstrate that the sample does not interfere with the ability of the test to detect endotoxin. Inhibition/enhancement testing should be preceded by preliminary testing in order to determine a concentration/dilution at which the product does not interfere. Provided that the preliminary testing is conducted properly, validation should be straightforward. Preliminary testing was discussed in the March 1996 issue of the LAL Update.

Regulatory Issues

The European Pharmacopoeia (EP), which only addresses the gel-clot method, states that the inhibition/enhancement test and routine release testing should be conducted at the maximum valid dilution (MVD). This is so that every test performed is a pass/fail test of the product at the endotoxin limit. A more practical approach has been advocated by the US Food and Drug Administration (FDA). The FDA has indicated preference for testing at a dilution less than the MVD, which is sufficient to overcome interference. The USP Bacterial Endotoxins Test chapter (BET) also addresses the gel-clot method only and states that the inhibition/enhancement test should be carried out at a dilution not to exceed the maximum valid dilution (MVD; for a discussion of the MVD see LAL UPDATE 13(4)). The advantage of working at less than the MVD is that product consistency/variability are monitored and problems can be identified before they cause the product to fail the test. The EP approach does not give any warning of a problem until the product fails the test.

The FDA Guideline on Validation of the *Limulus* Amebocyte Lysate Test (1987) (hereafter referred to as "the Guideline" in this article) addresses validation of release tests of finished product. It states that the inhibition/enhancement test should be performed on three lots of product. It does not specify the number of units per lot of drug product to be tested, but the sampling procedure given for routine testing may be adopted. Take a minimum of three vials from the beginning, middle and end of the lot. These vials may be pooled for testing (see LAL UPDATE, 14(1) p. 4, for a discussion of the implications of pooling vials and of an FDA recommendation on this subject). For products manufactured with different concentrations of active ingredient while the rest of the formulation remains constant, the Guideline states that the inhibition/enhancement test only needs to be performed on the highest and lowest concentrations. If there is no significant difference between these two, other concentrations do not have to be tested separately.

For medical devices, the Guideline clearly states the numbers of units that should be extracted and tested. For lot sizes of less than 30 devices, test two devices; for lot sizes of 30 - 100, test three devices; for lots of greater than one hundred devices, test 3% of the lot up to a maximum of 10 devices. The Guideline also allows different devices of similar chemical (i.e., material) composition to be grouped for inhibition/enhancement testing. The devices selected as representative of the group should be those with the largest surface area exposed to the body or the fluid for administration to a patient. There is no requirement to adjust the endotoxin limit for pooled medical device extracts. In fact, Section I in the FDA Guideline states that the endotoxin limit accounts for the effect of pooling. Also, endotoxin limits calculated using the formula in USP chapter 161 are not affected by the number of device extracts.

The USP BET requires that the product or product dilution contains no detectable endotoxin before the inhibition/enhancement test can be performed. The presence or absence of significant contaminant endotoxin is determined during preliminary testing. A rule of thumb is that the product solution tested for inhibition/enhancement should contain less than one quarter of the endotoxin limit.

In order to perform an inhibition/enhancement test, endotoxin is added to a single concentration/dilution of product and then tested. While the principle is the same for all LAL methods, there are differences, particularly between the gel-clot and other methods.

The Gel-Clot Method

For the gel-clot method, endotoxin is added to product on dilution of product to give concentrations of 2λ, λ, 1/2λ, 1/4λ, where λ is the LAL reagent sensitivity. This is commonly achieved by diluting endotoxin with product of the required concentration. These concentrations are tested in quadruplicate in parallel with the same endotoxin concentrations in water (tested in at least duplicate). The test includes negative controls (in at least duplicate) and unspiked product (in quadruplicate). The FDA Guideline refers to the USP BET, which states that the geometric mean (GM) endpoint of the standard series in water and that of the series in product must both be within a factor of two of the labeled sensitivity of the LAL reagent. Consequently they could differ by up to a factor of four from each other and still meet the USP requirement. The EP, in the Bacterial Endotoxins chapter requires that the GM endpoints of the series in product be within a factor of two of the determined sensitivity of the lysate in water. It does not specifically state that the endpoint in product must confirm the labeled sensitivity of the LAL reagent. If both conditions are met (the GM endpoints are within a factor of two of the labeled sensitivity and of each other), the requirements of both the USP and the EP are satisfied.

Example

Assume a label claim sensitivity (λ) of 0.125 EU/ml and a product MVD of 1:100 (this could be extended to 1:400 by using an LAL sensitivity of 0.03 EU/ml). Also assume that preliminary testing indicated inhibition at dilutions down to 1:4 and that this product is to be validated at a 1:25 dilution.

The results show that the label claim endpoint for the LAL reagent (0.125 EU/ml) has been confirmed within a factor of two in both water and in product. Also, the endpoint in product is within a factor of two of that in water. Thus, the requirements of both the USP and the EP are met.

STANDARDS IN WATER (EU/ML)

0.25	0.125	0.06	0.03	neg ctl
+	+	–	–	–
+	–	–	–	–

GM ENDPOINT = 0.18 EU/ML

STANDARDS IN SAMPLE (EU/ML)

0.25	0.125	0.06	0.03	sample (unspiked)
+	+	+	–	–
+	+	–	–	–
+	+	–	–	–
+	+	–	–	–

GM ENDPOINT = 0.11 EU/ML

Next Issue...
Inhibition or Enhancement Testing: Part 2

LAL-5000 Gains CE Mark in Europe

Associates of Cape Cod is pleased to announce the manufacture of the LAL 5000 Series II in Europe under the capable direction of The Orion Group. The European–manufactured machines will have the Conformité Européenne (CE) mark, which assures that they meet the CE radio frequency interference standards. With the coordinated manufacture of machines in both Europe and the U.S., ACC continues toward its goal of lowest cost supply and service. Future instruments manufactured in the United States will also meet CE standards.

ACC Renews Distribution Agreement with Seikagaku Corporation

Just prior to the International Endotoxin Meeting in Japan, ACC's President, Dr. Thomas Novitsky, renewed a distribution agreement with Japanese distributor, Seikagaku Corporation. Associates of Cape Cod is pleased to be represented by such a high quality organization and wish Seikagaku continued success in the LAL market!

International Study Establishes Single Endotoxin Standard

The World Health Organization (WHO) has accepted the same endotoxin preparation as the United States Pharmacopeia (USP) and the U.S. Drug and Food Administration (FDA) to be the official international endotoxin standard. This is extremely good news to all companies that market internationally and have struggled with the confusion and inconsistencies generated by the previous existence of several primary reference endotoxin standards for LAL testing.

Preparation 94/580 was established as the second International Standard (IS) for endotoxin by the Expert Committee on Biological Standardization of the World Health Organization in October 1996. The new IS is taken from a pool of two out of three sublots of endotoxin prepared at the National Institute for Biological Standards and Control (NIBSC) in the United Kingdom. The third sublot is the endotoxin designated EC–6 by the U.S. Food and Drug Administration which was accepted by the U.S. Pharmacopeial Convention as the U.S. endotoxin reference standard, Lot G, in 1995. Lot G/EC–6 had been assigned an activity of 10,000 Endotoxin Units (EU) per vial after a study comparing its activity against EC–5 in U.S. laboratories.

All three LAL methods were used to evaluate the performance of the candidate IS against the USP lot F/FDA EC–5 standard and against the existing IS and European and Japanese Pharmacopeial standards. On the basis of this study, preparation 94/580 was accepted as the new IS for endotoxin for all applications and was assigned an activity of 10,000 International Units (IU) per vial on the basis of its collaboration in terms of the FDA EC–5 standard: and therefore, one IU is one EU.

Given that the same preparation of endotoxin has been adopted by the FDA/USP and WHO, it is to be hoped that the EP and JP will also adopt this preparation. Since the EP standard was originally calibrated against the first IS, a standard for LAL gelation tests only, the adoption of the second IS as BRP–3 would make such a standard more appropriate for the turbidimetric and chromogenic methods.

ACC was particularly pleased to participate in the international collaborative study as one of the 26 laboratories in the 13 countries involved, and as the supplier of the common lysate. ACC was also involved in the FDA and USP studies that established the new lot as the FDA and USP reference endotoxin. We extend our congratulations to Dr. S. Poole, Dr. H.D. Hochstein (CBER, FDA), and Dr. L.V. Feyns (USP) for achieving the acceptance of the single, truly international, standard endotoxin.