LAL Update®

ASSOCIATES OF CAPE COD INCORPORATED

SEPT. 1997 / VOL. 15, NO. 3

Letter from the President

This issue contains an article by Michael Dawson covering the progress to date on the "harmonization" of LAL regulations world-wide. Although much has been accomplished, I think uniform procedures are still a few years away.

That we have come this far since the LAL test was first allowed by the FDA in 1977 is due to the hard work of many people. One of these, Dr. H. Donald "Don" Hochstein, has just retired from the FDA. To mark this occasion, I would like to acknowledge his accomplishments.

I have had the pleasure of knowing Don since I began my career with ACC in 1978. His knowledge and enthusiasm were instrumental in the acceptance of LAL by both industry and the FDA. Don's willingness to work with the LAL manufacturers and his experience with both the pyrogen test and LAL insured a smooth transition for pharmaceutical manufacturers. His work on endotoxin standards is directly related to the progress summarized in this UPDATE. I will miss my discussions with Don and his Washington area weather reports. Best wishes for an enjoyable retirement!

Sincerely,

Thomas J. Novitsky, Ph.D.

International Regulation of the LAL Test

By Michael E. Dawson Ph.D.

Includes the New Bacterial Endotoxins Chapter in the European Pharmacopoeia-Supplement 1998

If you have visited our web site (www.acciusa.com) and clicked on the Worldwide Distributors button, you will have seen the display of flags representing all the countries in which Associates of Cape Cod's products are available. This is a testament to the global acceptance of the LAL test. With acceptance have come regulations that govern the use of LAL for testing parenteral drugs and solutions, biological products and medical devices. Regulatory agencies in different countries have sometimes written their own regulations and in other cases have referred to the United States Pharmacopeia (USP) or the European Pharmacopoeia (EP). Inevitably, as the various pharmacopeia wrote monographs for the test, different test procedures were specified in the various monographs. For example, until recently, there were significant differences in the gel-clot procedures between the USP Bacterial Endotoxins Test (BET) chapter and the Japanese Pharmacopeia (JP) BET chapter. As a result of changes published in JP XIII, the requirements are now quite similar. Likewise the differences between the USP BET and EP Bacterial Endotoxins chapter have been reduced over recent years.

The JP is the lead pharmacopeia in a formal effort to harmonize the chapters on bacterial endotoxins testing. A revised EP Bacterial Endotoxins chapter has recently been published in the 1998 Supplement to the EP. It includes some significant changes from the 1997 chapter. The changes bring it into closer agreement with the JP chapter in that it includes details on endpoint chromogenic methods and kinetic turbidimetric and chromogenic methods. However, there are still some differences and it is likely to be some time before a single procedure has been agreed upon by all of the various pharmacopeia. In the mean time, companies have products that are being marketed in many countries, and frequently these markets are subject to different regulatory requirements. Manufacturers want to minimize the need for multiple tests in order to meet the different requirements; they want to develop a single procedure that will address all the requirements.

In addition to the endotoxins test chapters in the pharmacopeia, there are other important documents to be considered. These include: the United States Food and Drug Administration (USFDA) "Guideline on Validation of the Limulus Amebocyte Lysate test as an End-product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products and Medical Devices" (1987); the USFDA "Interim Guidance for Human and Veterinary Drug Products and Biologicals: Kinetic LAL Techniques" (1991);

(Continued on Page 4)

In This Issue...

International Regulation of the LAL Test 1
Comparison of Different Regulatory Requirements (Table) 2–4
Calendar 4

COMPARISON OF DIFFERENT REGULATORY REQUIREMENTS

DOCUMENT	USP BET	USFDA GUIDELINE/GUIDANCE	EP ET	EP: GUIDELINES	JP BET
Reference	USP 23	1987 Guideline and 1991 Guidance	2.6.14., EP-Supplement 1998	2.6.14., EP-Supplement 1998	JP XIII
TOPIC					
METHOD	Gel-clot. Other methods are acceptable if shown to comply with the requirements for alternate methods.	Gel-clot, endpoint chromogenic, and kinetic chromogenic, and turbidimetric methods.	Gel-clot limit limit test (the default) and assay, kinetic turbidimetric and chromogenic methods, and endpoint chromogenic method.	Gel-clot, endpoint chromogenic, kinetic chromogenic, and turbidimetric methods.	Gel-clot; turbidimetric and colorimetric (i.e. chromogenic) grouped as "photometric methods."
REAGENT	LAL of labeled sensitivity.	LAL licensed by CBER.	LAL regulated by competent authority with labeled sensitivity given in I.U./ml.	LAL or lysate from a closely related species such as *Tachypleus sp.*	LAL, TAL etc.,
STANDARD ENDOTOXIN	USP Endotoxin Reference Standard (RSE) or CSE of known potency (test 1 vial in quadruplicate).	RSE or CSE.	Endotoxin BRP (Biological Reference Preparation) calibrated in International Units (I.U.) against the WHO International Standard (IS) for Endotoxin.	Endotoxin BRP standardized against the WHO International Standard for Endotoxin (IS) with a potency in I.U./ml. CSE of known potency relative to the BRP or IS permitted.	Endotoxin Reference Standard (JP standard, though this is not stated). Endotoxin unit is expressed in EU (of JP standard, which was standardized against US RSE).
pH	Reaction mixture (sample + LAL) pH: 6.0–8.0. Adjust with NaOH, HCL or buffer if necessary.	Not specified.	Sample pH: 6.0–8.0. Adjust pH with 0.1 N NaOH or HCl or buffer if necessary.	States that pH of reaction mixture (sample + LAL) should be 6.0–7.5 and that sample pH should not be < 6.5.	Reaction mixture (sample + LAL) pH: 6.0–8.0. Can be adjusted with 0.1 N NaOH or HCl. Buffer may be used.
INITIAL TESTING: GEL-CLOT – CONFIRMATION OF LABELED SENSITIVITY OF LAL REAGENT.	Required. For at least 1 vial of LAL, test 2λ, λ, 1/2λ and 1/4λ (where λ (lambda) = labeled sensitivity) in at least quadruplicate. Confirm label claim within a factor of two.	Refers to USP for gel-clot. Gives standard curve requirements for chromogenic and turbidimetric methods.	Required. Test four replicate series of standard endotoxin to give 2λ, λ, 1/2λ, and 1/4λ. Confirm label led sensitivity within a factor of two.	States that it is important to follow the manufacturer's directions.	Required. Test 2λ, λ, 1/2λ and 1/4λ in at least quadruplicate. Confirm label led sensitivity claim within a factor of two.
INITIAL TESTING: ENDPOINT METHODS – LAL REAGENT QUALIFICATION	Not described.	Calculate linearity for standard endotoxin concentrations in at least quadruplicate, r value must be ≥ 0.980.	Test four replicate standard series of BRP dilutions to cover the range indicated by the manufacturer, including λ. Regressions line must have significant linearity and slope at 95% significance.	Not described.	Calculate linearity for at least 3 standard endotoxin concentrations in at least triplicate; r value must be ≥ 0.980.
INITIAL TESTING: KINETIC METHODS – LAL REAGENT QUALIFICATION	Not described.	At least 3 endotoxin concentrations in at least triplicate. If range exceeds 1 log, additional standards included to bracket each log increase in sensitivity range. |r| ≥ 0.980.	Test 2 replicate standard series of at least 4 concentrations of BRP, at least one conc. per log range. Regression line must have significant linearity and slope at 95% significance.	Not described.	At least 3 endotoxin concentrations in at least triplicate. If range exceeds 1 log, additional standards included to bracket each log increase in range. |r| ≥ 0.980.
INHIBITION OR ENHANCEMENT TEST: GEL-CLOT METHOD	Confirm labeled sensitivity in sample in quadruplicate at a dilution not to exceed the maximum valid dilution (MVD). Include standards in water in at least duplicate.	Refers to USP for gel-clot. Test at least 3 lots.	Compare labeled sensitivity in water and product in quadruplicate at a dilution not to exceed the MVD. Endpoints in product and water must be within a factor of two of each other. Neutralization, dialysis, ultrafiltration or chemical addition mentioned to overcome interference.	Notes the importance of preliminary testing. Does not clearly discriminate between preliminary tests and inhibition or enhancement(I/E) tests for validation. Mentions ultrafiltration for removal of interfering factors. Perform the I/E test on at least three production batches.	Confirm labeled sensitivity in sample in quadruplicate at dilution not to exceed the MVD. Include standards in water in duplicate.
INHIBITION OR ENHANCEMENT TEST: ENDPOINT METHODS	Not described.	Test sample in duplicate unspiked and spiked at 4λ. Spikes must be recovered within 75-125%. Test at least 3 lots.	Test sample at a dilution not to exceed the MVD (ELC/λ_m) unspiked and spiked at the mean of min. and max. standard series concentrations (λ_m or $\lambda_{m'}$). Spike must be recovered between 50 and 200%.	Not described.	Dilute sample to calculated concentration; dilution factor = endotoxin limit (in EU/ml)/m; m is the mid standard curve conc. Test sample unspiked and spiked. Spikes must be recovered within 75–125%.
INHIBITION OR ENHANCEMENT TEST: KINETIC METHODS	Not described.	Test sample in duplicate, unspiked and spiked at 0.1–0.5 EU/ml if PFC ≤ 1 EU/ml, or at 5 EU/ml if PFC>1 EU/ml, or at 4λ. Spikes must be recovered within 50–150%. Test at least 3 lots.	Test sample at a dilution not exceeding the MVD (ELC/λ_m or ELC/$\lambda_{m'}$) unspiked and spiked at a standard concentration from the middle of the standard series (λ_m or $\lambda_{m'}$). Measured spike concentration must be ≥ 50% of the nominal concentration.	Not described.	Dilute sample to calculated concentration; dilution factor = endotoxin limit (in EU/ml)/m; m is the mid standard curve conc. Test sample unspiked and spiked. Spikes must be recovered within 50–200%.

COMPARISON OF DIFFERENT REGULATORY REQUIREMENTS

TOPIC	USP BET	USFDA GUIDELINE/GUIDANCE	EP ET	EP: GUIDELINES	JP BET
Reference	USP 23	1987 Guideline and 1991 Guidance	2.6.14., EP-Supplement 1998	2.6.14., EP-Supplement 1998	JP XIII
TOPIC					
TEST PROCEDURE: GEL-CLOT METHOD	Add appropriate volume of LAL to sample. Incubate at 37 ± 1˚C for 60 minutes ± 2 minutes. Test in in duplicate with standards. Include negative controls, and 2λ positive product controls. Perform an assay by testing a geometric series of dilutions.	Does not give specifics. Refers to USP. Include negative controls, Standards or 2λ positive controls and 2λ positive product controls. A full standard series must be included with at least the first test of the day.	Add sample to lysate at 37˚C. Flexible regarding sample and reagent volumes, incubation period ("usually 20–60 min.") etc. Test sample at a dilution not exceeding the MVD in duplicate. Include negative controls. (A) Limits test: 2λ positive and positive product controls. (B) Assay: Include standard series, dilutions of sample from validated dilution and include 2λ spike of validated dilution.	Specifics not described. Includes details on endotoxin limits and determination of MVD.	Add 0.10ml sample/ standard to 0.10ml LAL. 1 hour incubation, 37 ±1˚C, 60 ±2 min. Test sample at the MVD. Include negative controls, 2λ positive controls and 2λ positive product controls. Standard series not required. Or perform assay using sample dilutions.
TEST PROCEDURE: ENDPOINT AND KINETIC METHODS	Not described.	Test sample at validated dilution, unspiked and spiked as for inhibition/ enhancement test. Positive control allowed in place of full standard series for kinetic methods. Data may be analyzed using archived curve provided positive control recovered within ± 25%.	Test sample at validated dilution, unspiked and spiked at λ_m (or $\lambda_{m'}$) as for inhibition/ enhancement test. Include three logrithmically equi-distant standards for kinetic methods, min. and max. standard concs. for the endpoint chromogenic method.	Specifics not described. Includes details on endotoxin limits and determination of MVD.	Include negative control and standard series. Test at validated dilution of samples, unspiked and spiked at m (where m = the endotoxin limit for that dilution).
INTERPRETATION: GEL-CLOT	For a valid test: negative controls must be negative, standards must confirm labeled sensitivity, positive product controls must test positive. To pass, the sample must test negative at a dilution not to exceed the MVD.	For a valid test: negative controls must be negative, standards must confirm labeled sensitivity or positive controls must be positive, positive product controls must test positive. Sample passes if it tests negative at a dilution not to exceed the MVD.	For a valid test: negative controls must be negative, positive product controls must be positive. (A) Limits test: Positive controls must be positive, Sample passes if it tests negative. (B) Assay: Standards must confirm label claim. Sample passes if GM conc. is < limit.	Specifics not described. Purposes of negative controls, positive controls and positive product controls are described. The test must be repeated at the MVD if result is positive at a dilution less than the MVD.	For a valid test: negative controls must be negative, standards must confirm labeled sensitivity or positive controls must be positive, positive product controls must test positive. Sample must contain < limit.
INTERPRETATION: ENDPOINT AND KINETIC METHODS	Not described.	Negative controls must not contain significant endotoxin. Standard curve must have |r| ≥ 0.980. If used, positive control must be within ± 25% of nominal value. Spikes must be recovered as for I/E test. Sample must contain < limit.	Result for negative controls must not exceed blank value at lysate qualification. Standard series must meet parameters for lystate qualification. Spikes must be recovered within 50-200%. Both replicates of sample must contain < λ_m (or $\lambda_{m'}$).	Specifics not described.	Negative controls must not contain significant endotoxin. Standard curve must have |r| ≥ 0.980. Spikes must be recovered within 50–200%. Sample passes if it contains less endotxin than the spike concentration (m).
RETESTS	Positives at less than the MVD may be retested at the MVD.	Provides for two; one to ensure that the test was not contaminated and one in case the sample was contaminated after collection.	Only allows a retest if one replicate of sample passes and the other fails.	1. Repeat tests permitted in case failure is due to faults in sample preparation, dilution or contamination by the analyst. 2. Positive gel-clot tests at dilutions less that the MVD should be repeated at the MVD.	Only allows a retest if one replicate of sample tests positive and the other tests negative.
MEDICAL DEVICES	USP Transfusion and Infusion Assemblies chapter <161>. Extract 3–10 devices at room temp for one hour starting with LRW at 37˚C. Test according to BET. Gives formula for endotoxin limit. Provides for MVD.	Flush with LRW for one hour at room temp; extract for 15 min. at 37˚C or one hour at room temp. Endotoxin limit 0.5 EU/ml for 40ml rinse. No formal provision for MVD. Validation to be performed in the lab that to performs end-product testing. Allows for other volumes with adjustment of limit.	Not described.	States that the endotoxin limit concentration (ELC) depends on K, M, and on how the extract/eluate is prepared. refers to "the specific monograph."	Not described.
RADIO-PHARMACEUTICALS	Limit=175 EU/whole body dose of the product at expiration.	Not described.	Not described.	Endotoxin limit (K) = 2.5 EU/kg/hr.	Not described.
DEPYROGENATION	Temperature "such as…250˚C or above for sufficient time."	Not described.	If necessary, equipment is treated to eliminate endotoxins.	Not described.	Usually heat at 250˚C for at least 1 hour.

COMPARISON OF DIFFERENT REGULATORY REQUIREMENTS

TOPIC	USP BET	USFDA GUIDELINE/GUIDANCE	EP ET	EP: GUIDELINES	JP BET
Reference	USP 23	1987 Guideline and 1991 Guidance	2.6.14., EP-Supplement 1998	2.6.14., EP-Supplement 1998	JP XIII
TOPIC					
MISCELLANEOUS		Assessment of the variability of the testing laboratory is specified before any official tests are performed. No guidance on how to do this is given.	Cautions that labware should be tested for interfering factors or adsorbance of endotoxins.	1. Substitution of the LAL test where a rabbit pyrogen test is specified in the product monograph requires validation (see I/E testing) with the agreement of the competent authority. If pyrogenic batches of product are available they should be tested by with the LAL test. 2. Cautions about adsorbance of endotoxins to containers.	Plastics should be tested for interfering effect.

International Regulation *(continued from front cover)*

and the non-mandatory "Test for Bacterial Endotoxins: Guidelines" that follows the EP Bacterial Endotoxins chapter in EP-Supplement 1998. The product inserts that are included with every shipment of Pyrotell, Pyrotell-T or Pyrochrome are sometimes overlooked as documents of regulatory importance. Inserts from USFDA licensed LAL manufacturers are USFDA approved documents as required by the Code of Federal Regulations. The procedure in the insert should be followed using the specified materials and supplies. A firm wishing to deviate from these procedures should validate the changes and be prepared to defend them. A practical reason for not deviating from the insert is that technical support and trouble shooting are very difficult if standard procedures are not followed.

The core of this article is a table showing the principal points of the major regulatory documents. For the gel-clot method, the differences between the documents are relatively minor. The gel-clot method is the only one for which there are detailed procedures in the official endotoxins test chapters in the USP, the EP and the JP. Because a common approach to gel-clot testing has evolved over the past 10 years, it should be possible to set up a single gel-clot test procedure that will satisfy most of the three pharmacopeia requirements. Where a difference is felt to be significant, the appropriate regulatory authority should be contacted in an attempt to resolve the discrepancy so that tests do not have to be repeated to satisfy a single condition.

There are significant differences for the photometric methods (both turbidimetric and chromogenic) between the requirements in the USFDA Guideline/Guidance and those of the EP and JP chapters. Some of these are a result of a difference in philosophy. The USFDA approach has been to perform a valid assay to quantify the endotoxin concentration in the product. The product passes if the measured concentration is less than the endotoxin limit. The EP/JP approach is more rigid and focuses more on the pass/fail decision with less emphasis on quantitation of the endotoxin concentration. However, with careful thought it is possible to define procedures that satisfy the intent of the various regulations. This does require a full understanding of the requirements. It is the aim of this article to assist with that understanding.

Caution: The most recent version of the original regulatory documents must be consulted before any changes in test procedures are adopted. This table is the author's summary of the current documents but it cannot be used or regarded as a substitute for the original documents.

CALENDAR

OCTOBER

October 21 – 22
LAL Methodology and Applications Seminar and Workshop
Buenos Aires, Argentina

NOVEMBER

November 3 – 5
Center for Professional Enhancement – LAL Testing: Drugs, Medical Devices, and Biotechnology
Directed by Dr. Michael E. Dawson
Amsterdam

November 10 – 12
PDA Annual Meeting
Philadelphia, PA

November 17 – 19
LAL Methodology and Applications Seminar and Workshop
Marriott Medical Center Hotel
Houston, Texas

ASSOCIATES OF CAPE COD, INC.
704 Main Street ▪ Falmouth, MA 02540

For customer service call
(800) LAL–TEST or (508) 540–3444

For technical service call
(800) 848–3248 or (508) 540–3444

Please visit us on the internet at
www.acciusa.com

©1997 Associates of Cape Cod, Inc.
All rights reserved.
Printed on recycled paper.