LAL Update

ASSOCIATES OF CAPE COD, INCORPORATED • VOLUME 19, NO. 2

Letter from the President

Dear LAL User:

On January 1, 2001, the United States and European Pharmacopeias revised versions of their endotoxin test chapters became official. The revisions are the long awaited attempt at harmonization. In this LAL UPDATE, Dr. Michael Dawson outlines the similarities and differences between the pharmacopeias. He concludes that although the documents are not identical, harmonization has been achieved.

This month also begins ACC's collection season, that, despite concerns over the potential decline of the US horseshoe crab population and growing demand for LAL, should satisfy our projected needs. We are also seeing significant progress on our new 80,000 square foot production and research facility. Occupancy is expected by the end of the year with production to follow in 2002. The new facility is designed to meet all of the latest FDA concerns regarding the manufacture of LAL and is the keystone of ACC's plan to establish itself as the unchallenged leader in endotoxin and glucan testing. The facility was also designed to accommodate the manufacture of our proprietary LAL alternative, which represents the future of endotoxin testing.

Another key part of ACC's plan is to continue to supply high quality reagents for all LAL methods, accessory reagents and supplies, as well as machines and software. The Pyros Kinetix tube reader is now available for sale as is the Platereader. Our new line of PyroClear plates and pipettes are also in stock. Finally, a White Paper has just been released outlining our development plan and commitment to Part 11 compliant Pyros software. Please contact our Technical Services department for more information.

Sincerely,

Thomas J. Novitsky, Ph.D.

New USP and EP Harmonized Endotoxins Test Chapters

by Michael E. Dawson, Ph.D.

Introduction

Both the United States Pharmacopeia (USP) and the European Pharmacopoeia (EP) published major revisions to the endotoxins test chapters in 2000. This was the result of a harmonization effort between the US, European and Japanese pharmacopeial organizations, which was lead by the Japanese Pharmacopoeia (JP). The chapters became official on January 1, 2001. This article does not address the harmonized version of endotoxins test in the JP, but it is expected to be very similar to the USP and EP chapters. An english language draft of the proposed chapter was published in JP Forum, 9 (4), 2000 with comments due by the end of November, 2000. The JP chapter will be reviewed in a future edition of the LAL Update.

The new chapters in the US and European pharmacopeia are very similar. There are some wording differences and a few minor concepts that are addressed in one document but not the other. When compared to the previous versions of the chapters there are some major changes. These are:

- The chapters give details for turbidimetric and chromogenic methods. This is new for the USP, not for the EP.
- The procedure for the gel-clot limit test does not require inclusion of a standard series, only a positive control.
- The USP chapter no longer contains reference to control standard endotoxins (CSEs). This omission has caused some concern among LAL users and is addressed in more detail below. In summary, it is our understanding that the USP had no intention of changing the status of CSE in the new chapter but felt that it was already addressed by general provisions of the USP.
- The EP chapter has eliminated references to λm and λm', simplifying the procedure.

- While not a change in the EP, the spike recovery range specified for positive product controls in the turbidimetric and chromogenic methods is 50-200%. This is different from the +/- 50% (that is 50-150%) given in the FDA Guidance on Kinetic LAL Methods and in Associates of Cape Cod's Pyrotell-T and Pyrochrome inserts. Until inserts are changed, the +/- 50% limits should be observed.

One point that should be noted is that the venerable gel-clot method is the "reference method" in most cases. It is the final arbiter in the case of a dispute unless another test method is specified in the product monograph. To the authors' knowledge, the only monographs specifying a particular LAL test method are those in the EP for Penicillin G and Penicillin G procaine. These specify an endpoint chromogenic test.

It is a testament to the success of the harmonization effort that it is possible to summarize the procedures in a single column in the table.

***ATTENTION*:** The table is the author's summary of the current documents and should not be used or regarded as a substitute for the official documents.

Control Standard Endotoxin (CSE)

The removal of reference to CSE from the USP Bacterial Endotoxins Test chapter was addressed in Pharmacopeial Forum 26(1), Jan-Feb, 2000, in which the proposed text of the harmonized chapter was published. In a preamble to the new text the USP stated: "The use of in-house standards shown to be equivalent to USP Reference Standards is permitted under the requirements for alternate methods in the General Notices. The control standard endotoxin (CSE) has thus been deleted because in-house standards have to be shown to be equivalent to the USP Endotoxin RS." In the General Notices section, on page 7 of USP 24, under Procedures, it is stated that: "Compliance may be determined also by the use of alternative methods, ..." and "Such alternative ... procedures or methods shall be validated." and ".. in the event of a dispute, only the result obtained by the procedure given in this Pharmacopeia is conclusive."

The term "in-house standards" in Pharmacopeial Forum suggests standards that are made up in individual laboratories. By contrast, the CSEs provided by the LAL manufacturers are widely used throughout the industry, as opposed to being used within one organization. As such they are more than in-house standards, but they have to be validated as alternatives to the USP Endotoxin Reference Standard.

For routine tests, alternate endotoxin standards which have been shown to be equivalent to the RSE can be used, provided that documentation of testing demonstrating that equivalence is available. Certificates of analysis (which give the potency of the CSE relative to the RSE) provide documentation of equivalence. There is a long history of the acceptance of certificates of analysis by the US FDA and regulatory agencies of other countries. Even before the publication of the new chapter, some companies performed testing on a limited number of lots of CSE to confirm that potencies given on certificates of analysis are correct. A few perform their own potency determination for every CSE/LAL lot combination. This is a decision for individual companies. In the case of the gel-clot test using a CSE potency taken from a certificate of analysis, every test in which label claim is confirmed supports the potency on the certificate.

It is important to recognize that the test procedures described in the USP are for "referee tests." These are the tests that are run in the event of a dispute regarding the concentration of analyte in a product. The procedures described are not written for routine use, though they are often assumed to be and are frequently used in that way.

Conclusion

The new endotoxins test chapters represent a significant improvement in the clarity and uniformity of these documents. They will undoubtedly simplify compliance for manufacturers whose products are sold in multiple countries. All of those who worked on this project in Europe, the United States and Japan are to be congratulated.

TABLE NOTES
(see pages 3 and 4)

1. The BRP is a harmonized standard and is the same endotoxin as the WHO International Standard and the USP Endotoxin Reference Standard (see LAL Update 15, no 4, Dec. 1997).
2. Gel-clot assay: It is recommended that the assay be performed if a product was validated at less than the MVD and a sample tested positive in a limit test. It passes if negative at the MVD in the assay. It is also recommended that the series of sample dilutions include the MVD.
3. Interfering factors: Lambda m and lambda m' have been removed from the EP. No validity requirements are given but negative controls should not contain significant endotoxin and standard curves must have lrl =/> 0.980. These are the requirements specified in the Test Procedure section.

TOPIC	COMMON PROCEDURE	DIFFERENCES BETWEEN USP AND EP	NOTE #
GENERAL			
REAGENT	LAL Reagent from *Limulus* or *Tachypleus.*	USP requires a reagent which has been prepared for use as an LAL Reagent. A footnote in the USP mentions glucans. EP requires reagent to be manufactured in accordance with the regulations of the "competent authority".	
METHOD	Gel-clot (limit test and assay); turbidimetric and chromogenic.	USP groups turbidimetric and chromogenic as "photometric methods" and notes that endotoxin concentrations are determined by interpolation from a standard curve. EP distinguishes between kinetic and endpoint methods.	
FINAL ARBITER	Gel-clot techniques are the ultimate arbiter unless otherwise indicated in the monograph.	USP allows for a gel-clot assay. EP specifies the limits test as the ultimate arbiter.	
APPARATUS AND GLASSWARE	Depyrogenate glassware etc. Suggests minimum of 30 min at 250^{o}C for dry heat. Plastics should be tested for contamination and interference.	A footnote in the USP addresses validation of dry heat sterilization and requires use of an LAL reagent with a sensitivity of at least 0.15 EU/mL.	
STANDARD ENDOTOXIN	USP and EP reference the USP Endotoxin RS and the WHO International Standards respectively - which are harmonized standards (see LAL Update 15, no 4, Dec. 1997).	USP specifies USP Endotoxin RS and describes reconstitution and mixing. Expires 14 days after reconstitution. Mix 3 minutes prior to use and for 30 s between dilutions. Do not store dilutions. No mention of CSE in the USP. Was mentioned when a draft was published in PF. CSE is allowed according to Alternate Methods. EP specifies a standard endotoxin that has been calibrated in International Units (I.U.) against the WHO International Standard for Endotoxin, e.g. endotoxin standard BRP (Biological Reference Preparation). EP states 1 I.U. = 1 E.U.	1
SAMPLE PREPARATION	Dissolve or dilute drugs. Other solutions may be used	USP states that extracts of medical devices should be prepared in LRW. EP does not mention medical devices.	
pH	Reaction mixture (sample + LAL) pH in the range specified by the LAL manufacturer. Says usually "applies" (sic) to products with a pH in the range 6.0 - 8.0. Can be adjusted with acid, base or validated buffer as recommended by the lysate manufacturer.		
MVD	MVD = Endotoxin limit x conc. of sample/λ.	Lambda (λ) is defined only as the labeled sensitivity in the text of the USP. It is defined as the lowest standard concentration used for photometric methods in Table 4. In the EP text, it is more broadly defined as either the labeled sensitivity or the lowest concentration of the standard curve.	
ENDOTOXIN LIMIT	Limit = K/M	A footnote in the USP gives details of limits for radiopharmaceuticals and product administered per m^2 of body surface	

TOPIC	COMMON PROCEDURE	DIFFERENCES BETWEEN USP AND EP	NOTE #
GEL CLOT			
CONFIRMATION OF LABELED LAL REAGENT SENSITIVITY	Perform for each lot of LAL or when conditions change. Follow LAL manufacturer's directions (e.g 0.10 mL sample/standard to 0.10 mL LAL; one hour incubation, 37+/-1°C, 60 +/- 2 min). Test 2λ, λ, 1/2 λ and 1/4 λ and negative controls in quadruplicate. Confirm labeled sensitivity, expressed in IU/mL, within a factor of two.	USP specifies at least one vial of LAL. EP does not mentioned numbers	
INHIBITION OR ENHANCEMENT TEST	Confirm label claim in quadruplicate using standard endotoxin in sample at a dilution less than the MVD. Confirm label claim in at least duplicate with a parallel series of standards in water. Use MVD if necessary. Interference may be overcome by treatment. Validate treatment by spiking sample with endotoxin, treating and testing for endotoxin recovery.	Note: Allows a 4x difference between standards and product. The former EP procedure did not allow this.	
LIMIT TEST	Test sample (treated as validated) at a dilution not greater than the MVD. Include negative controls, 2λ positive controls and 2λ positive product controls, all in duplicate. Standard series not required. Controls must be valid and sample must test negative to comply. Repeat the test at the MVD if the product tests positive at a dilution less than the MVD. Also repeat the test if one replicate of the sample tests positive and the other negative.	The USP states that the Limit Test should be used when a monograph contains a requirement for endotoxin limits. The non-mandatory EP Guidelines say that the gel-clot limit test is the "reference method" and is to be used unless otherwise indicated in the monograph. Also, other methods must be validated and shown to give results consistent with the reference method.	
ASSAY	To quantify endotoxin concentration. Test a series of sample dilutions of sample "not to exceed the MVD" both unspiked and spiked. Include a full series of standards. For a test to be valid: negative controls must be negative, standards must confirm labeled sensitivity and positive product controls must test positive. Sample meets the requirements if it contains < limit in the monograph.		2
PHOTOMETRIC METHODS	Methods are outlined. Tests are carried out at the incubation temperature recommended by the LAL manufacturer, usually 37+/-1°C.		
VERIFICATION CRITERIA FOR THE STANDARD CURVE	Calculate linearity for at least 3 standard endotoxin concentrations in at least triplicate. Absolute value of the correlation coefficient (lrl) value must be =/> 0.980. Follow manufacturer's instructions on method. For kinetic methods, if range exceeds 2 logs, include additional standards to bracket each log increase in range. lrl =/> 0.980.		
INTERFERING FACTORS	Test sample at a dilution not to exceed the MVD, unspiked and spiked with endotoxin to give a concentration at or near the middle concentration of the standard curve. There must be at least three concentrations for the standard curve. The added endotoxin must be quantified within 50-200% of the known added concentration after subtraction of any endotoxin in the unspiked sample. Include negative controls and test in at least duplicate.		3
TEST PROCEDURE	Same as test for Interfering factors.		
INTERPRETATION: ENDPOINT & KINETIC METHODS	Test must be valid: negative controls should not exceed the limit in the description of the LAL reagent used; for the standard curve, lrl =/> 0.980; the added endotoxin spike must be quantified within 50-200% (as for the interference test). Sample must contain less than the endotoxin limit when corrected for dilution and concentration.		

Finally!

Direct Measurement of (1-3)-β-D-Glucans

Associates of Cape Cod, Inc. is pleased to introduce **Glucatell™,** a rapid and ultra-sensitive assay kit that allows quantitation of (1-3)-β-D-Glucans down to picogram/ml levels. Glucatell™ kits are for use in a 96-well microplate format and are used for the detection and quantification of (1-3)-β-D-glucans in the following applications:

- **Mycology** - cell walls, fungemia and mycosis research
- **Food Quality Research** - mold exposure
- **Fluid Contamination** - culture media, buffers, reagents, biologicals and cellulose filtered solutions.
- **Environmental Air Quality** - sick building syndrome and workplace air analysis including agricultural product processing sites.
- **Material Quality Control** - bulk excipients, active ingredients
- **Investigation of LAL False Positives**

Glucatell is for Research Use Only

Catalog#	**Description**
GS001	Glucatell Kit - Kinetic Assay (110 determinations)
GS002	Glucatell Kit - Endpoint Assay (110 determinations)

For additional information or to place an order, call our Customer Support Center at 800-LAL-TEST

Under Construction

Our new 80,000 sf building is under construction and going strong. The state-of-the-art facility will allow us to expand our production and testing capabilities for our customers. At present, our administrative office are scheduled for move-in in November. Production staff and processes will move in 2002 once all validation work is complete. Watch our website at www.acciusa.com/happenings for new developments and building progress.

New Facility - Front elevation (May 15, 2001)

Organization Announcement

Effective 1 February 2001, Mr. Tony Coyle assumed the new position of General Manager Europe. This is our fastest growing market. In this new role, Tony will be responsible for all of our European operations including ACCI UK, Pyroquant, and our distributors in Europe, Africa, the Middle East, and Central Asia.

Effective 1 February 2001, Mr. Mark Childs responsibilities at ACCI UK were expanded to Manager ACCI UK. He will be responsible for the general management and sales for ACCI UK.

Congratulations to Tony and Mark for their hard work and dedication and special thanks to Dr. Peter Weidner for his continued commitment to ACC. They have been instrumental in our success.

CALENDAR OF EVENTS

MAY 2001

May 21 - May 23
ASM Annual Meeting
Orange County Convention Ctr
Orlando, FL
Booth #611

JULY 2001

July 16 - July 18
Biomedical Focus
Touchstone Energy Place, RiverCentre
St. Paul, MN

July 17 & 18
Introductory Topics Course
Sheraton Hyannis Resort
Hyannis, MA

July 19
Laboratory Course
Sheraton Hyannis Resort
Hyannis, MA

July 20
Advance Topics Course
Sheraton Hyannis Resort
Hyannis, MA

SEPTEMBER 2001

September 18 & 19
Introductory Topics Course
Holiday Inn Select
Bloomington, MN

September 20
Laboratory Course
Holiday Inn Select
Bloomington, MN

September 21
Advance Topics Course
Holiday Inn Select
Bloomington, MN

DECEMBER 2001

December 3 - December 7
PDA Annual Meeting
Marriott Wardman Park Hotel
Washington, DC
Booth #603 & 605

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

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

Please visit our website!
www.acciusa.com

Printed on recycled paper.

ASSOCIATES OF CAPE COD INCORPORATED®

Creating New Horizons in Endotoxin Testing

LAL Update®
VOLUME 19, NO. 2