ASSOCIATES OF CAPE COD INCORPORATED
Specialists in Endotoxin and Glucan Detection

Controls for Photometric Tests: Implications for Low Capacity Test Systems

by Robert Porzio and Michael Dawson, Ph.D., RAC

April, 2009

LALUpdate

volume 25, number 1

Letter From The Editor

Dear LAL User:

The bacterial endotoxins test chapters in the United States, European and Japanese pharmacopeia specify particular controls for the test. Consequently, a minimum number of samples (i.e. reaction tubes, wells or channels) is needed in order to run tests that are compliant with the pharmacopeia. This LAL Update looks at the impact of the pharmacopeial requirements upon the minimum sample capacity of test systems used for photometric tests.

While not addressed in the pharmacopeia, the FDA guidance documents on LAL testing provide for use of archived standard curves, provided that certain prerequisites are met and that appropriate controls are included in tests that use archived curves to determine endotoxin concentrations. The implications of these provisions on test systems are also discussed.

We hope that those users in the Northern hemisphere are enjoying a pleasant spring and that those of you south of the equator had a good summer.

With best wishes,

Michael Dawson, Ph.D., RAC

Controls for Photometric Tests: Implications for Low Capacity Test Systems

by Robert Porzio and Michael Dawson, Ph.D., RAC

Summary

Instrumentation for photometric endotoxin tests must have a capacity for at least 12 samples in order to meet the requirements of the pharmacopeial endotoxins test chapters. Small test systems with a low capacity, including those that rely on archived curves, cannot accommodate the controls specified in the pharmacopeia or guidance documents.

Introduction

The minimum sample numbers for photometric test systems are described in this article. These are dictated by the requirements for specific controls given in the bacterial endotoxins test (BET) chapters in the United States, European and Japanese pharmacopeia and by the recommendations made in United States Food and Drug Administration's (FDA) guidance documents.

System Test Capacity

The pharmacopeial BET chapters[1, 2, 3] specify the numbers of samples* required for different types of test. In each of the three types of test described the minimum number of samples is **twelve**. (The details of the requirements are given in the box accompanying this article.) Consequently, in order to conduct testing in compliance with the BET chapters, the capacity for instrumentation used should be at least twelve samples. This applies regardless of whether samples are tested in microplate wells, reaction tubes or other channels for introducing the sample into the reader.

In the FDA guidance documents similar recommendations are given. An additional provision is made that describes the use of archived standard curves. An archived standard curve is a previously determined set of parameters that define the standard line, which is then used to determine endotoxin concentrations in subsequent tests. Prior to using archived standard curves consistency of standard curves must first be demonstrated in the test laboratory. The FDA "Interim Guidance for Human and Veterinary Drug Products and Biologicals" of 1991 (which has been incorporated into the online version of the 1987 Guideline[4]) specifically refers to "your" laboratory, clearly indicating that consistency should be shown by the user, not some other laboratory, such as that of the reagent manufacturer. The Guidance describes constructing the archived curve from data from standard curves tested over three consecutive days. Consequently, the capacity for twelve samples capacity is necessary so that a full standard series can be tested, even if archived standard curves are to be used in the future.

The guidance documents specify that standard (positive) controls should be included with the test; these serve to verify the validity of the archived standard curve. The test should also include negative controls, sample and positive product controls, all in duplicate, resulting in a minimum capacity for eight samples when an archived standard curve is used.

In order for an archived standard curve to be considered valid for use, the standard control must be quantified within +/- 25% of the known concentration of the standard. This depends on highly reproducible tests and may be difficult to achieve. Failure to recover the standard control results in an invalid test and the test must be repeated. Without a standard control there is no check that the archived standard curve is valid.

The provision for use of archived curves is only made in the sections on Routine Testing in the guidance documents. Therefore, when verifying reagent performance or performing the test for interfering factors (inhibition/enhancement testing), a full standard curve should be included with the test, again requiring a minimum of twelve samples.

Conclusion

Unless the test system being used for photometric test has a capacity for at least twelve samples, it is not possible to conduct testing that is in strict compliance with the pharmacopeial bacterial endotoxins test chapters. The same number is also required for compliance with the 1991 FDA Guidance document.

* In this article the term "sample" refers to a single replicate of any type of material being tested, including negative controls, endotoxin standards, unknown samples, positive product controls and standard controls.

[1] Bacterial Endotoxins Test. Chapter <85>. USP 31, 98-102, United States Pharmacopeial Convention, Rockville, MD, 2007.

[2] Endotoxins Test, European Pharmacopeia 6.0, chapter 2.6.14, 182-189.

[3] Bacterial Endotoxins Test, Japanese Pharmacopeia XV, chapter 4.01, 77 – 81. (http://jpdb.nihs.go.jp/ - jp15e/JP15.pdf, accessed 11/12/08).

[4] http://www.fda.gov/cder/guidance/old005fn.pdf (accessed 11/12/08)

ASSOCIATES OF CAPE COD INCORPORATED
Specialists in Endotoxin and Glucan Detection

Corporate Headquarters
Associates of Cape Cod, Inc.
124 Bernard E. Saint Jean Drive,
East Falmouth, MA 02536
T (508) 540–3444
F (508) 540–8680
www.acciusa.com

UK Office
Associates of Cape Cod Int'l Inc.
Deacon Park, Moorgate Road,
Knowsley, Liverpool L33 7RX
United Kingdom
T (44) 151-547-7444
F (44) 151-547-7400

European Office
PYROQUANT DIAGNOSTIK GmbH
Opelstrasse 14,
D-64546 Morfelden-Walldorf,
Germany
T (49) 61 05-96 10 0
F (49) 61 05-96 10 15

Controls for Photometric Endotoxin Tests

The endotoxin test chapters in the United States Pharmacopeia (USP), the European Pharmacopoeia (EP) and the Japanese Pharmacopoeia (JP), which were harmonized in 2001, all require inclusion of specific controls with each test. Negative controls and a series of endotoxin standards are required for all tests, all tested in duplicate.

Negative controls consist of samples of LAL reagent water containing no detectable endotoxin to which LAL reagent is added. Their purpose is to assure that the test system does not give a signal in the absence of endotoxin and to verify that the reagents are not contaminated.

The series of standards consists of at least three known concentrations of endotoxin reference standard (RSE). From the response to each for the known standard concentrations, a standard line is constructed (usually mathematically) from the system output (optical density or onset time). This line is used to quantify the endotoxin concentration of samples relative to the standards.

An additional control is required for each sample tested, whether in the test for interfering factors or in a routine test. This is a positive product control (PPC) consisting of a known concentration of standard endotoxin added to the sample. The endotoxin concentration measured in the PPC must be within 50-200% of the known concentration. A measured PPC concentration outside of this range indicates that the sample interferes with the detection of endotoxin and the test is invalid.

Without the specified controls, an endotoxin test cannot meet the requirements of the pharmacopeial.

Three types of test are described in the pharmacopeial endotoxins test chapters, two of which come under the heading Preparatory Testing. The third type is the test itself, described under the heading Procedure for the Photometric Techniques. The three types of test are summarized in Table 1.

Table 1: The Three Types of Photometric Test in Pharmacopeial Endotoxin Test Chapters

Test Type	Purpose	Test requirements:	
		Test set-up	Results
Preparatory Tests			
1. Verification of Criteria for the Standard Curve	Verification of test performance	At least three concentrations of RSE in triplicate and negative controls **Number of samples: ≥12**	The absolute value of the correlation coefficient, Irl, for the standard curve must be ≥ 0.980
2. Interfering Factors Test for the Photometric Techniques	Demonstrate that the sample does not interfere with detection of endotoxin	At least three concentrations of RSE and negative controls For each sample, test in parallel with a PPC. All standards, samples and controls are tested in at least duplicate. **Number of samples: ≥12**	The value Irl for the standard curve must be ≥ 0.980. The measured concentration of added endotoxin must be within 50-200% of the nominal concentration
Procedure for the Photometric Techniques (i.e. Routine Tests)			
3. Sample test	Test for endotoxin	Same as the Interfering Factors Test above. **Number of samples: ≥12**	The value Irl for the standard curve must be ≥ 0.980. The measured concentration of added endotoxin must be within 50-200% of the nominal concentration Negative controls meet requirements in the description of the LAL reagent.

Questions to Consider Regarding Archived Standard Curves and Low Capacity Test Systems

1. Can the performance of LAL reagent lots be verified in a low capacity test system without the ability to reproduce a standard curve as specified in the USP/EP/JP?

2. Can an archived standard curve be used to perform the interfering factors test to validate the method in accordance with USP/EP/JP requirements?

3. Can routine endotoxin testing be performed per the USP/EP/JP using an archived standard curve?

4. Can routine endotoxin testing be performed per the FDA Guidance documents using an archived standard curve without Standard Controls and Negative Controls?

5. Can I perform a series of tests in a low capacity test system to meet the requirements for testing sample, PPC, and positive and negative controls?

6. Can the accuracy and precision of the archived standard curve be verified?

If you wish to discus any of these questions, please call your local representative or our Technical Service personnel.

ASSOCIATES OF CAPE COD INCORPORATED
Specialists in Endotoxin and Glucan Detection

Corporate Headquarters
Associates of Cape Cod, Inc.
124 Bernard E. Saint Jean Drive,
East Falmouth, MA 02536
T (508) 540–3444
F (508) 540–8680
www.acciusa.com

UK Office
Associates of Cape Cod Int'l Inc.
Deacon Park, Moorgate Road,
Knowsley, Liverpool L33 7RX
United Kingdom
T (44) 151-547-7444
F (44) 151-547-7400

European Office
PYROQUANT DIAGNOSTIK GmbH
Opelstrasse 14,
D-64546 Morfelden-Walldorf,
Germany
T (49) 61 05-96 10 0
F (49) 61 05-96 10 15

LAL Training Workshop 2009 Schedule & Fees

To register for a course, contact our Customer Service department at (800) 525-8378 - in North America, or download the registration form online at www.acciusa.com/lal/training/index.html. For information on training courses outside the United Sates, contact your local office or distributor.

APRIL 21-23 - Marriott, Miami, FL - *Registration Deadline: April 7, 2009*

April 21	LAL Methodology Background Course	**$425**
April 22	Laboratory Training*	**$500**
April 23	In-Depth Topics Course	**$475**

JUNE 23-25 - ACC Facility, Falmouth Technology Park, East Falmouth, MA - *Registration Deadline: June 16, 2009*

June 23	LAL Methodology Background Course	**$425**
June 24	Laboratory Training*	**$500**
June 25	In-Depth Topics Course	**$475**

SEPTEMBER 15-17 - Marriott, Bloomington, MN - *Registration Deadline: September 1, 2009*

September 15	LAL Methodology Background Course	**$425**
September 16	Laboratory Training*	**$500**
September 17	In-Depth Topics Course	**$475**

OCTOBER 6-8 - ACC Facility, Falmouth Technology Park, East Falmouth, MA - *Registration Deadline: September 29, 2009*

October 6	LAL Methodology Background Course	**$425**
October 7	Laboratory Training*	**$500**
October 8	In-Depth Topics Course	**$475**

NOVEMBER 3-5 - La Jolla Marriott, La Jolla, CA - *Registration Deadline: October 23, 2009*

November 3	LAL Methodology Background Course	**$425**
November 4	Laboratory Training*	**$500**
November 5	In-Depth Topics Course	**$475**

*When registering for laboratory training, please select one of the following:

❍ Gel-clot Lab ❍ Photometric Lab (plate reader) ❍ Turbidimetric Lab (tube reader)

For more information, contact Marketing at marketing@acciusa.com. If you would like to register for one of the training workshops, please call 1-800 LAL TEST (525-8378) or download the 2009 registration form at http://www.acciusa.com/lal/training/schedule.html.

2009 Tradeshow/Conference Schedule

Date	LAL Tradeshows	Location
April 26-29, 2009	ANNA	San Diego, CA
April 20-24, 2009	PDA Annual Meeting	Las Vegas, NV
May 3 - 9, 2009	ARVO	Fort Lauderdale, FL
June 13 - 16, 2009	IACP	Washington, DC
September 14-18, 2009	PDA/FDA	Washington, DC
October 5-8, 2009	PDA's Annual Global Microbiology Conference	Bethesda, MD
October 21 - 22, 2009	Medical Design & Manufacturing Exposition	Minneapolis, MN
November 5-6, 2009	Bacterial Endotoxin Summit	Newark, NJ
November 9 - 11, 2009	AAPS Annual Meeting	Los Angeles, CA

ASSOCIATES OF CAPE COD INCORPORATED
Specialists in Endotoxin and Glucan Detection

Corporate Headquarters
Associates of Cape Cod, Inc.
124 Bernard E. Saint Jean Drive,
East Falmouth, MA 02536
T (508) 540–3444
F (508) 540–8680
www.acciusa.com

UK Office
Associates of Cape Cod Int'l Inc.
Deacon Park, Moorgate Road,
Knowsley, Liverpool L33 7RX
United Kingdom
T (44) 151-547-7444
F (44) 151-547-7400

European Office
PYROQUANT DIAGNOSTIK GmbH
Opelstrasse 14,
D-64546 Morfelden-Walldorf,
Germany
T (49) 61 05-96 10 0
F (49) 61 05-96 10 15