

The Bacterial Endotoxins Test: A Practical Approach

Karen Zink McCullough

**PDA
Bethesda, MD, USA
DHI Publishing, LLC
River Grove, IL, USA**

10 9 8 7 6 5 4 3 2 1

ISBN: 1-933722-49-5

Copyright © 2011 Karen Zink McCullough

All rights reserved.

All rights reserved. This book is protected by copyright. No part of it may be reproduced, stored in a retrieval system or transmitted in any means, electronic, mechanical, photocopying, recording, or otherwise, without written permission from the publisher. Printed in the United States of America.

Where a product trademark, registration mark, or other protected mark is made in the text, ownership of the mark remains with the lawful owner of the mark. No claim, intentional or otherwise, is made by reference to any such marks in the book.

While every effort has been made by the publisher and the author to ensure the accuracy of the information expressed in this book, the organization accepts no responsibility for errors or omissions. The views expressed in this book are those of the editors and authors and may not represent those of either Davis Healthcare International or the PDA, its officers, or directors.



This book is printed on sustainable resource paper approved by the Forest Stewardship Council. The printer, Gasch Printing, is a member of the Green Press Initiative and all paper used is from SFI (Sustainable Forest Initiative) certified mills.

PDA

4350 East West Highway
Suite 200
Bethesda, MD 20814
United States
www.pda.org/bookstore
301-986-0293

Davis Healthcare International Publishing, LLC

2636 West Street
River Grove
IL 60171
United States
www.DHIBooks.com

CONTENTS

Foreword	xiii
Author's Preface	xv
Glossary	xix
I Discovery and Acceptance of the Bacterial Endotoxins Test	I
<i>James F. Cooper</i>	
Discovery	I
Preparation of <i>Limulus</i> Amebocyte Lysate (LAL)	3
Origin of LAL methods	3
Comparison of LAL and pyrogen tests	4
Commercialization of LAL reagent	5
Symposia at Woods Hole	6
Acceptance	6
Pyrogen and LAL tests at the FDA	7
Industry acceptance of LAL methods	7
The LAL-Test Guideline	8
Revolutionizing the validation of depyrogenation processes	9
LAL Reactive Glucan (LRG) challenges specificity	9
Evolution of the compendial Bacterial Endotoxins Test (BET)	10

Summary	10
References	11
About the Author	13
2 Understanding Reaction Basics	15
<i>Michael E. Dawson</i>	
Introduction	15
The LAL Clotting Reaction	15
The recombinant Factor C assay	22
Endotoxin	24
Glucans	25
Conclusion	26
References	26
About the Author	28
Appendix	29
3 Constructing and Interpreting Standard Curves for Quantitative BET Assays	31
<i>Karen Zink McCullough</i>	
The Endpoint Assay	33
The Kinetic Assay	36
Accuracy	39
Anatomy and Attributes of a Standard Curve	40
Slope	40
y-intercept	41
Correlation coefficient	41
Coefficient of variation	42
Analysis: Effects of Standard Curves on the Accuracy of Data	43
Scenario 1 — effect of changes in slope	45
Scenario 2 — effect of changes in y-intercept	48
Scenario 3 — effect of changes in %CV	50
Scenario 4 — effect of non-linearity	52
Alternate regression analysis	56
Discussion	57
Conclusion	60
References	61
About the Author	62

4	Qualifying the Laboratory	63
	<i>Ronald N. Berzofsky</i>	
	Equipment	64
	Qualifying Incubators	65
	Qualifying Timers	70
	Qualifying Ancillary Supplies	71
	Qualifying Procedures	74
	Inhibition/enhancement	74
	Storage of samples and standards	75
	Qualifying Reagents	76
	The LAL reagents	76
	Endotoxins	77
	Buffers and solutions	78
	Qualifying the analyst	80
	Dilutions	80
	Conclusions	82
	References	83
	About the Author	83
5	Calculating Endotoxin Limits, Maximum Valid Dilutions and Minimum Valid Concentrations	85
	<i>Karen Zink McCullough</i>	
	Endotoxin Limits	85
	Endotoxin limits for small volume parenteral drugs and biologicals	86
	Endotoxin limits for large volume parenterals	92
	Endotoxin limits for radiopharmaceuticals	93
	Endotoxin limit for drugs administered per square meter of body surface	94
	Setting endotoxin limits for a product	95
	Endotoxin limits in test solutions	96
	Maximum Valid Dilution (MVD)	97
	Minimum Valid Concentration (MVC)	99
	Summary	101
	Problem Set	102
	References	110
	About the Author	113

6	Assigning Endotoxin Limits to Noncompensial Articles	115
	<i>Michael E. Dawson</i>	
	Conclusion	129
	References	129
	About the Author	130
7	Applying USP Test Requirements: Medical Devices	131
	<i>Marilyn J. Gould</i>	
	The Challenge	131
	USP and Other References Pertaining to Testing Devices	132
	Historical Perspectives — How Much Endotoxin is Pyrogenic?	133
	A Reference Standard Endotoxin and Threshold	
	Pyrogenic Dose	134
	Comparing the 1987 FDA Guideline Device Limits with	
	Drug or Biologics Limits	135
	Intraethical Endotoxin Limit	135
	USP <161> Specifies LAL Reagent Water (LRW) as the	
	Extraction Medium	136
	Situations When a Solvent Other than Water	
	May Be Required	137
	Qualify the Extraction Medium	138
	Sample Sizes May Vary with Lot Sizes	138
	USP Equation to Calculate Limits for the BET	139
	Extraction Volumes Less than 40 mL/Device	140
	Extraction Volumes Greater than 40mL/Device	141
	Find a Convenient Volume Other than 40 mL/Device	141
	Maximum Valid Dilution (MVD)	142
	What is a Device for Purposes of Calculating Limits?	143
	Critical Surfaces	143
	Before Testing a Device for the First Time	144
	What Extraction Volume Should be Used?	149
	What if the Ideal Extraction Volume Exceeds the	
	Calculated Maximum Volume?	150
	Multiple extractions in the same volume	150
	Concentrate the extract	150
	Extraction Containers	152
	Extractions — Physical Considerations	152
	Challenge and Recovery	153
	Handling Devices to Set Up Extractions	154
	Steps to Perform a USP Test on a Medical Device	155
	One Final Note	157

References	158
About the Author	161
8 Verifying USP Test Methodology	163
<i>Karen Zink McCullough</i>	
Spiking	167
Double-double method	168
Hot spike	169
Test Method Development	170
Calculation of the endotoxin limit	171
Calculation of the MVD and/or MVC	173
Preliminary testing	174
Inhibition/Enhancement, or Verification of the Suitability of the Test Method	181
Suitability testing for gel clot	182
Suitability testing for quantitative tests	185
Some Questions Regarding Suitability Testing	186
Chapter Questions	187
Appendix. Example Template for a Gel Clot Suitability Study	188
References	192
About the Author	193
9 Resolving Test Interferences	195
<i>John Dubczak</i>	
Inhibition	196
Inhibition due to pH	197
Inhibition due to high osmolarity	200
Inhibition due to chelating agents	200
Inhibition due to Ca ⁺⁺ containing formulations	201
Inhibition due to protease inhibitors	202
Inhibition due to heavy metals	202
Inhibition due to detergents	204
Inhibition due to proteins	204
Inhibition due to liposomes	205
Enhancement	206
Enhancement due to beta 1-3 glucans	206
Enhancement due to serine proteases	210
Enhancement due to detergents	210
Conclusion	212
References	212
About the Author	214

10	Performing Routine Tests	215
	<i>Alan Baines</i>	
	Introduction	215
	Equipment and materials	217
	Sampling	218
	Sample containers and accessories	218
	Sampling Water for Injection (WFI)	219
	Sampling incoming raw materials	221
	In-process samples	222
	Drug product sampling	225
	Pooling	225
	Storing endotoxin standards and test samples	227
	Establishing a Testing Routine	228
	Sources of error	228
	Assay prerequisites	229
	Controls	233
	Negative control	233
	Positive Product Control (PPC)	233
	Method 1: “Double-double” method	234
	Method 2: “Hot spike” method	234
	Standard series (gel clot) or standard curves (quantitative tests)	236
	Sample preparation	237
	Assay set-up — gel clot	238
	Gel clot limits test	238
	Gel clot assay	240
	Assay set-up: quantitative tests	241
	Standard curves in routine testing	242
	Archived standard curves	243
	Product standard curves	244
	Positive product controls and routine testing	245
	Data Analysis and Interpretation	248
	Calculating and reporting results for the gel clot limits test	249
	Gel clot assay	250
	Quantitative tests	252
	Converting EU/ml to EU/unit of drug product	252
	Robotics and Routine Testing	253
	Reporting Results	253
	Header section	254
	Standards	255
	Sample results	257
	Masking test results	259

Reviewing results	259
Alert and action limits	260
OOS and re-testing	261
Trending	262
Appendix 1 Effect of high background on PPC Recovery	264
Appendix 2 Robotics	266
References	269
About the Author	270
11 Investigating Out-of-Specification (OOS)	
BET Results	271
<i>Karen Zink McCullough</i>	
1987 FDA Guideline	272
Current Thinking: FDA's 2006 OOS Guidance	274
Principle 1. The laboratory may not test a product into compliance	274
Principle 2. Test results may not be averaged into compliance	275
Principle 3. Investigations into OOS results must be thorough, unbiased, scientific, timely and well documented	276
Principle 4. Investigations, where appropriate, must include associated batches of product	278
SOPs for Investigating BET OOS Results	280
Phase I Laboratory Investigations: Identifying Critical Points in the Lab	283
Creating the OOS Checklist: Fault Tree Analysis	285
Retesting	292
Beyond the Laboratory: Phase II Investigation	292
Thoughts on the Pretest from the Beginning of this Chapter	294
Summary	295
References	295
About the Author	295
Appendix. Basic checklist for Investigating BET OOS Results	296
12 Structuring a Deyrogenation Study	299
<i>John Dubczak</i>	
Elements to a Deyrogenation Process Qualification	300
Endotoxin Indicators and Their Use	303
Dry Heat Pyrogenation	305
Dry Heat Process Qualification Studies	306
Cleaning/Rinsing Deyrogenation Process Qualification	306
Equipment cleaning/depyrogenation	307

Container/closure rinsing depyrogenation	308
Depyrogenation in Biotechnology	310
Ultrafiltration	311
Electrostatic attraction via charge modified media	312
Affinity chromatography	313
Other Means of Destroying Endotoxin	314
Acid and base hydrolysis	314
Oxidation	314
Conclusion	315
References	315
About the Author	317
13 Setting Alert and Action Limits	319
<i>Ronald N. Berzofsky</i>	
Process Capabilities vs. Product Specifications	319
Standard Deviation vs. Percentiles	321
Probability and an Unexpected Event	326
Control Charts	328
Steps in Calculating Alert and Action Events	329
About the Author	332
14 Identifying Critical Control Points in Manufacturing	333
<i>Karen Zink McCullough</i>	
The Manufacturing Process	334
The Product Formulation	337
HACCP	338
HACCP Step 1. Conduct a hazard analysis	339
HACCP Step 2. Identify critical control points (CCP)	339
Process step: receipt and testing of raw materials	341
Process step: sterile filtration	342
Process step: depyrogenation	343
Process step: QC testing	343
HACCP Step 3. Establish control limits	344
Process step: testing of raw materials	344
Process step: depyrogenation	345
Process step: QC testing	345
HACCP Step 4. Establish monitoring procedures	346
Process step: raw material testing	346
Process step: depyrogenation	347
Process step: QC testing	347
HACCP Step 5. Establish corrective actions	347

FMEA	348
HACCP Step 6. Establish verification procedures	356
HACCP Step 7. Establish record-keeping and documentation procedures	356
Summary	357
References	357
About the Author	359
15 Auditing the BET Laboratory	361
<i>Karen Zink McCullough</i>	
The Audit Cycle	364
Planning	365
Preparing an audit checklist	367
Preparation	369
Execution	373
Reporting	374
Monitoring	375
Summary	378
Appendix. Sample audit plan/checklist	379
References	382
About the Author	383
16 Regulatory Summary	385
<i>Karen Zink McCullough</i>	
Index	399

FOREWORD

Scott Sutton

It is an honor to have been asked to write the foreword to this important book on the Bacterial Endotoxins Test. This volume is significant not only because of the timing of the work, coming as the field is in a state of flux with new technologies and new regulations, but also because of its practical, bench-level focus.

There are several books available on the Bacterial Endotoxins Test. Some approach it from an ecological perspective, focusing on the horseshoe crab and the impact that farming is having on its population. Others address the topic from a clinical and chemical perspective, heavy on theory and academic considerations. These are valuable contributions to the literature and their review is recommended for anyone who wishes a broad grounding in the development and science of the test. However, they are not directed specifically at the needs of the lab worker in regulated industries. In fact, this book is one of a very limited number of technically competent works directed to the QC lab worker in any topic and for that reason alone warrants a place on the serious quality professional's bookshelf.

Even beyond this consideration, it has to be noted that it is difficult to bring a diverse group of workers together and produce a book that is readable and technically challenging at the same time. The top-rank authors assembled herein have produced such a book, and done it without yielding to the temptation of promoting their own products. This is especially noteworthy in that most of the authors work for companies that are in direct competition with each other, and are generally not shy about disagreeing with each other. The discussions in this book are remarkable in the absence of commercial promotion — science and technical methodology take center stage throughout.

Enjoy the time you spend with this book. It won't really matter if you are looking for a historical perspective on the development of the test or an explanation of exactly why the y-intercept is an important consideration of the standard curve, you will almost certainly learn something new about the bacterial endotoxin test and the role it plays in QC operations in pharma, biopharma, medical devices and all healthcare-related regulated industries

Scott Sutton, Ph.D.
Microbiology Network, Inc.
April, 2011

AUTHOR'S PREFACE

Welcome.

The goal of this book is to provide sensible and useful information for those who are responsible for the performance and interpretation of the Bacterial Endotoxins Test (BET). The concept was to create a user-friendly reference tool for BET analysts, laboratory managers and regulatory/compliance specialists that is part lab manual, part textbook, part tutorial, and part non-commercial consultant.

For those laboratories new to BET, this book lays a solid foundation by providing information and tips for qualifying the laboratory, calculating endotoxin limits, verifying USP test methodology, resolving test interferences and performing routine tests for parenteral drugs, biologics and medical devices.

For those laboratories that are experienced in BET, this book builds on basic testing requirements by discussing topics such as

setting endotoxin limits for noncompendial articles, structuring a depyrogenation study, setting action and alert limits and identifying endotoxin-specific critical control points in manufacturing.

The content of this book represents an amazing 200+ author-years of experience in the field. With many thanks to Alan Baines, Ron Berzofsky, Jim Cooper, Mick Dawson, John Dubczak and Marilyn Gould for their extraordinary contributions to this effort, their remarkable perspective and their expert sage advice, I hope that the information in this book will help everyone to take a practical approach to the Bacterial Endotoxins Test.

Karen Zink McCullough
Whitehouse Station, NJ
April, 2011