MICROBIAL RISK AND INVESTIGATIONS



Edited by Karen Zink McCullough and Jeanne Moldenhauer

CONTENTS

I	INTRODUCTION	I
	Karen Zink McCullough and Jeanne Moldenhauer	
	References	4
	About the Authors	4
2	CONDUCTING MICROBIAL DEVIATIONS AND INVESTIGATIONS	7
	Frank Settineri	
	Introduction	7
	ls it Real?	9
	Are Marketed Products Safe?	13
	Summary	21
	Conclusion	21
	What's the Risk?	23
	What's the Root Cause?	30
	Process flow	33
	Corrective and Preventive Actions (CAPA)	36
	CAPA Effectiveness	39
	Summary	40
	References	42
	About the Author	44

iii

SUCCESSFUL INVESTIGATIONS	45
Scott Sutton	
Introduction	45
Difficulties in Phase I Microbiological Investigations	48
Time	48
Laboratory variability	49
Common Mistakes in Conducting the Phase I	
Microbiology Investigation	51
The red truck fallacy	52
Testing to compliance	52
Training to exhaustion	53
Send the test to a comptent laboratory	54
One size fits all	55
Preparing for a Successful Investigation	56
The SOP system — procedures and well-designed	
data sheets	57
Retains and aging plates	60
Conducting a Successful Laboratory Investigation	60
Preliminary laboratory data review	60
Laboratory investigation	63
Closing the laboratory investigation	67
Corrective Action Plan	68
Investigating Specific Test Failures	69
Antimicrobial efficacy tests	69
Bacterial endtoxin tests	70
Environmental monitoring excursions	70
In-process tests — raw material and pre-sterilized	
bulk bioburden	72
Media fill events	73
Microbial limits tests	73
Sterility tests	74
Water systems testing	74
References	75
About the Author	76

iv

3

	Contents	v
4	THE MICROBIOLOGIST'S TOOL BOX	77
	Hilary Chan, Lynn Johnson, and Jill Larivee	
	Introduction	77
	Risks and Consequences of Microbial Contaminiation	
	in a Non-sterile manufacturing environment	78
	Anatomy of a Contamination Response Team	79
	Root cause analysis	81
	Remediation/control phase	82
	Contamination Support: What the Microbiologist	
	Brings to the Table	83
	Trending of microorganisms	84
	Risk assessment: a microbiologist's	~
	retrospective perspective	86
	Rapid microbiological methods	88
	Be Prepared: Build your Knowledge Base for Enhanced	0.2
	Contamination Responsiveness	93 93
	What is a microbiologist's contamination control kit?	93 94
	Contents of a microbiologist's contamination control kit Building a base of knowledge: prospectively gathering data	9 4 99
	Practical Applications of the Microbiologist's Contamination	,,
	Control Toolkit: Experimental Studies	105
	Building the study foundation: basic microbiological	105
	preparation techniques	105
	Microbial survival studies	103
	Summary	130
	References	131
	About the Authors	136
		150
5	QUALITY METRICS	139
	Karen Zink McCullough	
	Background	139
	Meaningful Metrics for Microbiological Data Deviations (MDD)	142
	Example Microbiological data deviations: OOS tests	145
	Example 2 MDDs: Out of limits results	152
	Example 3 MDDs: Out of Trend (OOT) results	154
	Example 4 MDDs: invalid test results	159
	Summary	163
	References	164
	About the Author	167

ANTIMICROBIAL EFFECTIVENESS TESTING (AET)

6	ANTIMICROBIAL EFFICACY TESTING IN		
	RISK ASSESSMENT	171	
	Phil Geis		
	Introduction	171	
	AET	173	
	Challenge microorganisms	174	
	Culture	175	
	Inoculum preparation	176	
	Manufacturing	177	
	In-Use	179	
	Drugs	179	
	Cosmetics	183	
	Household and industrial products	185	
	Summary	187	
	References	188	
	About the Author	198	

OBJECTIONABLE ORGANISMS

7 **OBJECTIONABLE ORGANISMS IN NON-STERILE PHARMACEUTICAL DRUG PRODUCTS: RISK ASSESSMENT AND ORIGINS OF** CONTAMINATION 201 David Roesti Introduction 201 Evaluation of Objectionable Microorganisms in Non-Sterile Pharmaceutical Products 203 General note 203 Example of a procedure for evaluating microbial growth in non-sterile products 203 Isolate characterization 207 Isolate bioload 208 The route of administration 210 Nature of the product 210 Intended recipient 211 212 Trending and manufacturing process Sources of Objectionable Microorganisms 213

Personnel	214
Environment	218
Raw materials	223
Water	225
Conclusion	228
References	220
About the Author	230
About the Author	233

vii

237

Contents

MICROBIAL LIMITS TEST

8 MICROBIOLOGICAL OUT-OF-SPECIFICATION AND OUT-OF-TREND INVESTIGATIONS IN NON-STERILE PHARMACEUTICAL MANUFACTURING — A CONTRACT LABORATORY PERSPECTIVE Bick lakober

Nex Jakober	
Introduction	237
Types of OOT and OOT	240
OOS, OOT and Objectionable Microorganisms	241
Investigations	243
Case Study	251
Results and conclusion of case study	254
References	255
About the Author	257

STERILITY TESTING

9	INVESTIGATING STERILITY TEST FAILURES	261
	Tim Sandle	
	Introduction	261
	Sterility Test Failure Investigations	262
	Immediate Actions	263
	Conducting Investigations	264
	Test laboratory	265
	Manufacturing	269
	Sterility Test and Process Area Link	273
	Genotypic microbial identification	274
	Re-testing the Finished Product Batch	274

Concluding Sterility Test Failure Investigations	275
Product Impact Assessment	276
Conclusion	278
References	279
Appendix: Sterility Test Failure Investigation Checklist	281
About the Author	288

10CONDUCTING STERILITY TEST
INVESTIGATIONS USING THE FDA MODEL291

Jeanne Moldenhauer	
Introduction	291
Sterility Testing Methods	292
Compendial Guidance on Sterility Test Investigations	293
FDA Guidance on Sterility Test Investigations	294
When a Sterility Test Failure is Identified	295
Procedures for Conducting the Investigation	296
Assessing the Validity of the Sterility Test Result	297
Conducting the Investigation	298
Identification of the sterility test contaminant	299
Review of laboratory test results and deviations	300
Microbial monitoring of the production area environment	302
Microbial monitoring of personnel	305
Product presterilization bioburden evaluation	305
Production records review	305
Review of the manufacturing history	306
Product Impact Analysis	306
Concluding the Investigation	307
Determining the Final Product Disposition	308
References	308
About the Author	309

ENVIRONMENTAL MONITORING

DETERMINING THE PRODUCT RISK WHEN	
CONTAMINATION OCCURS IN	
ENVIRONMENTAL MONITORING	313
Jeanne Moldenhauer	
Introduction	313
Investigation Expectations	314
	CONTAMINATION OCCURS IN ENVIRONMENTAL MONITORING Jeanne Moldenhauer Introduction

viii

Risk Assessments	315
Other Concerns with Contamination Events	321
The production of toxins	321
Closely related organisms	322
How much contamination is too much	322
Inability to identify the contaminant	322
Changes to Bergey's Manual	324
The Problem of "Ones"	324
Conclusion	325
References	325
About the Author	326

Contents

ix

12 LEVERING RISK ASSESSMENTS IN ENVIRONMENTAL MONITORING INVESTIGATIONS 327

Karen Ginsbury Introduction 327 Risk Management and the Quality System 329 The PA in CAPA 329 The Environmental Control Program — Proactive Quality Management 333 The environmental control strategy — "plan" of P-D-C-A cycle Risk mitigation and communication 333 Environmental Monitoring — Proactive Quality Management 334 "Check" of P–D–C–A cycle 334 Data Trending 338 Microbiological Data Deviations and Environmental 339 **Monitoring Excursions** Investigations: problem solving cycle The CA in CAPA 339 361 Knowledge Management and Lessons Learned Updating the risk assessment post investigation and improving the control strategy More PA in CAPA 361 References 364 About the Author 364

13	MICROBIAL INVESTIGATION CASE STUDIES	365
	Ken Muhvich	
	Introduction	365
	Background	366
	Case Studies — Failures to React to Environmental	
	Monitoring Excursions	370
	Case study #1 — Batch sterility failure	370
	Case study #2 — Batch sterility failure	371
	Case study #3 — Critical surface sample excursion	372
	Case study #4 — False–positive sterility tests	373
	Case study #5 — Batch sterility failure	374
	Case study #6 — Sterility test failure	375
	Case study #7 — Environmental monitoring excursions Case study #8 — Environmental monitoring excursions	377
	Case study #8 — Environmental monitoring excursions	378
	Case study #9 — Bulk solution bioburden excursion	379
	Case study #10 — Over action environmental data	
	excursion	381
	Analysis of EM Results — Important Questions to Answer	382
	References About the Author	383 384
		507
14	ASSESSMENT OF ENVIRONMENTAL	
	MONITORING DATA IN SUPPORT OF	
	ASEPTIC MANUFACTURING	385
	Veronica Marshall, David Correal, Mulyadi Setiawan	
	and Karen Zink McCullough	
	Introduction	385
	Current Environmental Monitoring Guidances: Viables	387
	Data	389
	The Dilemma	392
	Patterns of contamination	393
	Contamination recovery rates	396
	A Possible Compromise Program	399
	Investigations	401
	Summary	409
	References	409
	About the Authors	410

	Contents	xi
15	INVESTIGATIONS FOR PERSONNEL MONITORING	413
	Anne Marie Dixon	
	Background	413
	Qualification	415
	Case Study #I	416
	Exit monitoring	419
	Case Study #2	419
	Contamination Investigations — Cleanroom	422
	Case Study #3	422
	Test protocol	424
	Walking	426
	Sit/stand	429
	Door entrance	431
	Waist turning activity	434
	Curtain entrance	436
	Summary of case study #3	439
	Summary	440
	References	440
	About the Author	441

16 REAL-TIME RISK ASSESSMENT CONTROL STRATEGY

443

Sean Toler	
Real-time Risk Assessment Shaped from Quality by Design	443
QbD Risk Assessments to Develop RTRA	446
QbD Design Space Impact on RTRA	449
QbD Control Strategy of RTRA	449
Continuous Improvement from RTRA	454
Implementing the RTRA	458
Microbiologist Development for RTRA	47 I
Cultural Improvement from RTRA	474
Summary	475
References	476
About the Author	256

17	MOLD CONTAMINATION	48 I
	Brian G. Hubka and Jeanne Moldenhauer	
	Introduction	481
	What is the Concern with Mold Contamination?	483

Pathogenicity	483
Mycotoxin production	484
Allergic reactions	485
Invasiveness of mold	486
Criteria for mold growth	486
Damaging effects of mold	488
Expectations for cleanrooms	488
Other health concerns	488
Examples of Mold Contamination Events	489
The New England Compounding Center (NECC)	489
Warning Letter CBER-12-07	491
Warning Letter 13-ATL-17	491
Warning Letter WL-320-14-13	492
Microbial contamination of syringes	493
Conducting the Investigation	493
Investigating environmental monitoring excursions	494
Specific Considerations in Looking for Mold Contamination	496
Facilities	496
Equipment surfaces	497
HVAC equipment and performance	498
Airflows and pressurization	498
Environmental considerations	498
Cold storage areas	498
Gowning and changing areas	499
Hidden molds	499
Other contaminated surfaces	499
Supplies	499
Corrective Actions	500
Changing from Corrective Actions to Preventative Actions	501
Conclusion	502
References	502
About the Authors	504
MICROBIAL EXCURSIONS IN CLEANROOMS	507
Jim Polarine and Carol Bartnett	

Introduction	507
Cleanroom Monitoring	508
Non-viable monitoring	509
Viable monitoring	510
Causes of Microbial Excursions	512
Personnel and practices	512

18

Facility design and condition	516
Cleaning and disinfection	520
Equipment/raw materials/consumables transfer (ingress)	525
Investigating Microbial Excursions	528
Conclusion	533
References	534
About the Authors	538

Contents

xiii

54 I

MEDIA FILLS

19 FAILURE INVESTIGATIONS IN RESPONSE TO ADVERSE ASEPTIC FILLING SIMULATION RESULTS

Tony Cundell	
Introduction	541
Industry Practice	542
Regulatory Guidance	542
Evolution of the Media Fill Acceptance Criteria	549
Patterns of Microbiological Growth in Soybean Casein	
Digest Medium	550
Patterns of the Turbid Vial Distribution Within the Media Fill	55 I
Laboratory Investigations of Turbid Vials	552
Origin of Microorganisms Isolated from Failed Media Fills	553
Implications of the Human Microbiome Project to	
Pharmaceutical Microbiology	553
Manufacturing Investigations of Turbid Vials	555
Conclusions	557
References	557
About the Author	559

ENDOTOXIN INVESTIGATIONS

INVESTIGATION OF ENDOTOXIN OUT-OF-	
SPECIFICATION AND UNEXPECTED RESULTS	563
James F. Cooper	
Overview	563
Pyrogen Outbreaks of the Past	564
Pyrogenic outbreak related to Active Pharmaceutical	
Ingredient (API)	565
	SPECIFICATION AND UNEXPECTED RESULTS James F. Cooper Overview Pyrogen Outbreaks of the Past Pyrogenic outbreak related to Active Pharmaceutical

Endotoxin OOS related to equipment and sampling	567
Aseptic meningitis from contaminated intrathecal drug	567
Endotoxin OOS caused by faulty component	568
Errant positive BET on surgical eye solution	569
Results that are not Definable as an OOS Event	569
Hot well	570
Glucan interference	570
Low LPS recovery	571
Non-robust kinetic BET conditions	572
Robust Kinetic BET Conditions Minimize Investigations	572
Summary	573
References	575
About the Author	577

WATER SYSTEMS

21	INVESTIGATION OF MICROBIOLOGICAL EXCURSIONS DURING A WATER SYSTEM	
	VALIDATION	581
	Karen Zink McCullough	
	The Company	582
	The Product	582
	The Company's User Requirement Specification for	
	"Clean Water"	582
	The System	583
	The Validation Strategy	585
	PQ Phase I (PQ-I)	585
	PQ Phase 2 (PQ-2)	586
	PQ Phase 3 (PQ-3)	586
	The Data	587
	The Investigation Strategy	590
	The bones	591
	Sanitization	591
	Sampling and sampling containers	592
	Testing	593
	Water quality	594
	Hardware	595
	Assessment	596
	Conclusion	597
	References	598
	About the Author	598

Contents

PARTICULATES

22	PARTICULATE MATTER IN INJECTABLE	
	DRUG PRODUCTS	601
	Stephen E. Langille	
	Introduction	601
	Classification and Sources of Particulate Matter	602
	Clinical Effects of Injected Particulate Matter	605
	Route of administration	606
	Size and shape	607
	Number	610
	Composition	611
	Patient population	615
	Relevant Regulations and Standards	617
	Continuing Efforts	623
	Conclusion	624
	References	625

MICROBIAL IDENTIFICATIONS

23 INVESTIGATING MICROBIAL IDENTIFICATION RESULTS

Jeanne Moldenhauer	
Background on Microbial Identifications	640
Selecting the System for Use	641
How to Distinguish between Systems for Specific Types	
of Organisms	645
Taxonomic Issues	647
Typical Problems in Identifications	650
Organism preparation for testing	651
Use of similarity numbers	652
Phenotypic methods	652
RiboPrinter® methods	652
Verification of microbial identification methods	654
Difficulties in identification	654
Making the right assumptions	655
Conclusions	655
References	655
About the Author	658

639

STERILIZATION SUPPORT

24	ADDRESSING DEVIATIONS IN BIOLOGICAL	
	INDICATOR POPULATION COUNTS AND	
	D-VALUE ANALYSIS	671
	Jeanne Moldenhauer and I.J. Pflug	
	Introduction	671
	Regulatory expectations for microbial count and	
	resistance testing	673
	United States Pharmacopeia (USP) Compendial requirements	674
	Other guidance documents	675
	Deviations in Confirmation of Eumeration (Population) counts	676
	Performing D-Value Analysis	678
	Deviations when Confirming D-Values	680
	Use of a qualified laboratory	681
	Qualified personnel	683
	Verification of microbial counts	683
	Verification of resistance values	683
	Equipment comparisons	684
	Qualification of media	684
	Media supplements	685
	рН	685
	Organism strain, type and purity	685
	Heat resistance test method reviews	686
	Shipping conditions	687
	Technical information packs	687
	Conclusion	687
	References	688
	About the Author	691
	Appendix: The D-Value in 2003, and some of its History	692
	History of the D-Value	692
	General Comments about the D-Value	694
	Application of the D-Value Concepts to Other Systems	
	such as Ethylene Oxide, Hydrogen Peroxide, Low-Temperature	
	Steam Formaldehyde, Radiation, etc.	696
	Hydrogen Peroxide	697
	Literature Cited	699

	Contents	xvii
25	INVESTIGATING POSITIVE BIOLOGICAL INDICATORS OR INSUFFICIENT KILL OF INDICATORS IN STERILIZATION STUDIES	701
	Jeanne Moldenhauer	
	Background	702
	Biological indicators	702
	Regulatory actions taken for Bls	705
	Useful Tasks Prior to the Actual Testing of the Bls	706
	Sterilization Modeling and how it can be used with Investigations	708
	Dry heat sterilization	710
	Gas sterilization	712
	Radiation sterilization	712
	Survivor and kill time evaluations	713
	Considerations when a BI does not Yield the Expected Results	714
	Calculation of the probability of a positive BI	714
	Ensuring the BI challenge is appropriate for the process	716
	Failure to Deliver the Sterilization Process to the BI	717
	Moist heat	717
	Gas sterilization cycles	720
	Vapor sterilization cycles	720
	BI Heat Resistance is Affected by Substrates	720
	Resolving Issues in the Verification of Performance Standards Discrepancies in the enumeration of population	721
	(control) counts	721
	Discrepancies in thermal death time analysis (D-value)	723
	Other Considerations for Recovery of Aberrant BI Results	724
	Steam traps	724
	Other typical investigation parameters	724
	Conclusion	725
	References	725
	About the Author	729

RAPID METHODS

26	6 CONDUCTING INVESTIGATIONS	
	RAPID MICROBIOLOGY METHODS	733
	Jeanne Moldenhauer	
	Introduction	733
	Rapid Methods — Why use them?	733
	Automated Methods	735

Using Rapid Microbiological Methods to aid in	
Conducting Investigations	735
Narrowing Down Areas of Contamination	736
Case study	736
Case study	737
Evaluating the Effectiveness of Corrective Actions	737
Case study	737
Personel Gowning and Aseptic Behavior	738
Identification of Microorganisms	738
Conducting Investigations for Issues with RMMs	739
Case study	739
Where do you Start when the Results Indicate Contamination?	740
Typical Microbiological Investigations	741
Viability Methods	742
Case study	743
Higher counts	744
Case study	744
Viability Based Methods not Recovering all Organisms	745
Inability to Identify Microorganisms from the Contaminant	745
Laser Based Systems	746
Growth-Based Technologies	746
Sterility Investigations	747
General Issues with RMM Equipment	747
Contamination of RMM Equipment	747
Conclusion	748
References	748
About the Author	749

CONTAMINATION RISK EVALUATION

27	CONTAMINATION RISKS AND THE PATIENT Mark Hunter, Michelle Luebke, and Mark Pasmore	753
	Patient I	754
	Patient 2	754
	Infection	755
	Sepsis	757
	Signs and symptoms of infection/sepsis	758
	Patient risk factors	761
	Infection/sepsis: the perfect storm	765
	Investigation: the causal continuum	767
	Intrinsic continuum	769

xviii

770 Extrinsic continuum Manufacturing contamination impacts on patients 776 Microbial Contamination in the Manufacturing Environment 777 Non-sterile, terminally sterilized, aseptically filled 779 Proliferation 781 781 Ingress Biofilms as an elevated risk 782 Conclusions 783 References 784 About the Authors 790

Contents

28		
	ASSESSMENT	793
	Tim Sandle	
	Introduction	793
	Sources of Contamination	796
	The Regulatory Focus on Quality Risk Management	798
	Objectives of Risk Assessment and Risk Management:	
	The Key Concepts	800
	Contamination Control Risk Assessment	803
	Methods for prospective risk assessments	803
	Simple risk assessment tools	811
	Assessing retrospective contamination events	828
	Conclusion	841
	References	842
	About the Author	845

Index

847

xix