



Technical Report No. 69

Bioburden and Biofilm Management in Pharmaceutical Manufacturing Operations



PDA Bioburden and Biofilm Management in Pharmaceutical Manufacturing Operations Technical Report Team

Authors and Contributors

Vince Anicetti, Coherus Biosciences (Team Co-leader)
Marc Mittelman, Ph.D., Exponent (Team Co-leader)
Catherine Adley, Ph.D., University of Limerick
Peter Amin, Ph.D., U.S. FDA
John Arigo, Ph.D., U.S. FDA
Hal Baseman, Valsource, LLC
John Paul Bevel, Teva Pharmaceutical
Lucia Clontz, Ph.D., Xellia Pharmaceuticals
Sven Deutschmann, Ph.D., Roche Diagnostics GmbH
Rebecca A. Devine, Ph.D., Consultant to the Biopharmaceutical Industry
Mark Fornalik, Industrial Biofouling

Patricia Hughes, Ph.D., U.S. FDA
Anastasia Lolas, Visionary Pharma Consulting LLC
Randa Melhem, Ph.D., U.S. FDA
Peter Noverini, Azbil North America — BioVigilant Divison
Mark Pasmore, Ph.D., Baxter
T.C. Soli, Ph.D., Soli Pharma Solutions, Inc.
Paul Sturman, Ph.D., Center for Biofilm Engineering
Kalavati Suvarna, Ph.D., U.S. FDA
Tyler Tsang, Genentech
George Verghese, STERIS Corporation
Carmen M. Wagner, Ph.D., Strategic Compliance International, Inc.

DISCLAIMER: The content and views expressed in this technical report are the result of a consensus achieved by the authoring task force and are not necessarily views of the organizations they represent.

Bioburden and Biofilm Management in Pharmaceutical Manufacturing Operations

Technical Report No. 69

ISBN: 978-0-93-945976-6

© 2015 Parenteral Drug Association, Inc.

All rights reserved.



Table of Contents

1.0 INTRODUCTION	1	4.2.6 Materials.....	22
1.1 Scope and Purpose	1	4.2.6.1 Raw Material Sources	23
2.0 GLOSSARY OF TERMS	3	4.2.6.2 Raw Material Handling	23
3.0 OVERVIEW OF BIOBURDEN AND BIOFILMS	6	4.2.6.3 Material Container Handling	23
3.1 Bioburden Generation	6	4.3 Cleaning and Sanitization Program.....	23
3.2 Chemistry of Biofilm Formation	7	4.3.1 Types of Residues	24
3.3 Growth and Survival	8	4.3.2 Cleaning Processes.....	24
3.4 Microbial By-Products	8	4.3.2.1 Water Flush or Rinse	24
3.4.1 Endotoxins and Other Cell-Wall		4.3.2.2 Sanitization	24
Components.....	8	5.0 BIOBURDEN AND BIOFILM DETECTION AND	26
3.4.2 Exotoxins and Other By-Products	8	CHARACTERIZATION	26
3.5 Impact of Fluid Flow on Biofilms.....	9	5.1 Sampling Points and Frequency.....	26
3.6 Biofilm-Remediation Considerations	11	5.1.1 Upstream Production.....	26
4.0 DESIGN, CONTROL, AND PREVENTION		5.1.2 Downstream Processing.....	27
CONSIDERATIONS	13	5.1.3 Monitoring Strategy	27
4.1 Overview of Microbial-Control Strategy.....	13	5.1.4 Process Sampling	27
4.1.1 Quality System.....	14	5.1.5 Equipment Sampling	28
4.1.2 Risk Assessment	14	5.2 Sampling Strategy	28
4.1.2.1 Ingress of Microorganisms	14	5.3 Microbial Levels and Limits	28
4.1.2.2 Proliferation of Microorganisms.....	14	5.3.1 Environmental Contamination	
4.1.2.3 Persistence of Microorganisms	15	Recovery Rates	29
4.2 Design Considerations	15	5.4 Recovery and Analysis of Biofilms.....	30
4.2.1 Facility Design.....	15	5.4.1 Biofilm Sampling	30
4.2.1.1 Construction Materials	15	5.4.2 Biofilm Analyses	31
4.2.1.2 Cleaning Program	15	5.5 Microbial By-Product Detection	33
4.2.1.3 Environmental Controls.....	16	5.5.1 Endotoxins	33
4.2.1.4 Workflow	16	5.5.2 Other Microbial By-Products	34
4.2.1.5 Waste	17	5.6 Alternative Microbial-Detection Methods	34
4.2.2 Equipment Design.....	17	5.6.1 Rapid Microbial Methods (RMMs).....	34
4.2.2.1 Soft Part Management.....	17	5.7 Microbial Test Method Validation.....	36
4.2.2.2 Leak Control.....	18	5.7.1 Method Suitability	36
4.2.2.3 Automation.....	18	5.7.2 Alternative Method Validation.....	37
4.2.2.4 Cleaning.....	18	5.7.2.1 Microscopy	37
4.2.2.5 Equipment Hold Time and Storage	18	5.7.2.2 Spectroscopy	37
4.2.3 Water Systems and Gas		5.7.2.3 Chromatography	37
Utilities Designs	19	5.8 Approaches to Laboratory Investigations	37
4.2.3.1 Water Quality.....	19	5.8.1 Launching Investigations	37
4.2.3.2 Water System Sanitization	19	5.8.2 Investigation Outcomes	38
4.2.3.3 Hose and Valve Maintenance	20		
4.2.3.4 Process Gases	20		
4.2.4 Manufacturing-Process Design.....	20		
4.2.4.1 Single-Use Technology	21		
4.2.4.2 In-Process Limits	21		
4.2.4.3 In-Process Hold Times	21		
4.2.5 Personnel	22		

6.2.1.3	Review of Laboratory Environmental and Test Controls	41	7.1.3	The Investigation	48
6.2.1.4	Microbial identification	41	7.1.4	Root and Probable Causes	49
6.2.2	Root Cause Determination of Contamination.....	41	7.1.5	CAPAs	49
6.2.2.1	Risk Assessment.....	41	7.1.6	Verification of Effectiveness.....	49
6.2.2.2	Investigation of the Contamination Source	42	7.1.7	Lessons Learned	49
6.2.2.3	Investigation Considerations.....	43	7.2	Scenario 2: Biofilm in WFI System	49
6.2.2.3.1	Species of Contaminant(s).....	43	7.2.1	System and Process Description	49
6.2.2.3.2	Association with Recent Changes to the System or Past Problems	43	7.2.2	The Event.....	50
6.2.2.3.3	Supplemental Nonroutine Sampling	43	7.2.3	The Investigation	50
6.3	Remediation.....	43	7.2.4	Root and Probable Causes	50
6.3.1	Issues to Consider in Biofilm Removal.....	43	7.2.5	CAPAs	50
6.3.2	Cleaning and Sanitization Methods and Parameters.....	44	7.2.6	Verification of Effectiveness.....	50
6.3.2.1	Cleaning Step(s) for Biofilm Removal ..	44	7.2.7	Lessons Learned	50
6.3.2.2	Achieving Microbial Kill after Cleaning	44	7.3	Scenario 3: Insufficient Hot Water Sanitization of a Cold WFI Loop	50
6.3.2.3	Rinsing of Cleaning Agents and Disinfectants.....	45	7.3.1	System and Process Description	50
6.3.3	Equipment Replacement or Design Improvement.....	45	7.3.2	The Event.....	51
6.3.4	Preventive Actions	45	7.3.3	The Investigation	51
6.3.4.1	Ineffective Routine Sanitization Parameters	45	7.3.4	CAPAs	52
6.3.4.2	Untreated Surfaces	45	7.3.5	Verification of Effectiveness.....	52
6.3.4.3	Revision of Cleaning and Sanitization Indicators or Frequency	46	7.3.6	Lessons Learned	52
6.3.4.4	Correction of Poor Equipment or Process Design.....	46	7.4	Scenario 4: Contaminated Purified-Water Loop Due to Open Point-of-Use Dead Leg	52
6.4	Monitoring CAPA Effectiveness	46	7.4.1	System and Process Description	52
6.5	Communication of Investigation Results and Actions.....	46	7.4.2	The Event.....	52
6.5.1	Reviews of Risk-Assessment Reports	46	7.4.3	The Investigation	53
6.5.2	Regulatory Submission Notification or Modifications.....	46	7.4.4	CAPAs	53
6.5.3	Notification of Customers and Suppliers about Changes	46	7.4.5	Verification of Effectiveness.....	53
7.0	CONTAMINATION SCENARIOS	48	7.4.6	Lessons Learned	53
7.1	Scenario 1: Biofilm in Biologics Purification Equipment.....	48	7.5	Scenario 5: Nonsterile Batch Contamination by an External Biofilm	54
7.1.1	System and Process Description	48	7.5.1	System and Process Description	54
7.1.2	The Event	48	7.5.2	The Event.....	54
8.0	APPENDIX I: BIOPROCESS UNIT OPERATIONS AND TYPICAL PROCESS CONTROLS AND DETECTION	56	7.5.3	The Investigation	54
9.0	APPENDIX II: TYPICAL BACTERIAL RESPONSE LIMITS FOR BIOPROCESSING	58	7.5.4	CAPAs	55
10.0	REFERENCES	59	7.5.5	Verification of Effectiveness.....	55

FIGURES AND TABLES INDEX

Figure 3.1-1	Bacterial biofilm on 316 stainless steel.. 6	Table 5.4.2-1	Biofilm Analysis Methods 32
Figure 3.1-2	Biofilm Formation and Generation..... 7	Table 5.4.2-2	Biofilm Analysis Techniques Using Removable Coupons 32
Figure 3.5-1	Fluid-Flow Velocity Profiles for Laminar (a) and Turbulent (b) Flow Conditions within a Pipe 10	Table 5.5-1	Possible Effects of Biofilm on Product..... 33
Figure 3.5-2	Laminar Boundary Sublayer Thickness in a Two-Inch Pipe..... 10	Table 5.5-2	Possible Effects of Biofilm on Process Performance 33
Fig 4.0-1	Effective (Risk-Based, Science-Based, and Proactive) Microbial-Control Program 13	Table 5.6.1-1	Current RMM Technologies 35
Figure 5.1-1	Microbial Contamination Control in Bioprocessing 26	Figure 6.0-1	Flow Diagram of Investigation and CAPA Steps for Effective Remediation 39
Table 5.4.1-1	Biofilm Recovery/Analysis Methods .. 31	Figure 6.2.2.1-1	Fault-Tree Analysis to Determine the Root Cause of a Contamination..... 42

1.0 Introduction

In the pharmaceutical industry, microbial-control issues are frequently cited in U.S. FDA inspectional observations and have resulted in recalls and/or medical shortages (1). In a review of more than 600 microbiology-related U.S. recalls of sterile and nonsterile products from 2004 to 2011, the majority resulted from a “lack of sterility assurance,” indicating a potential problem with the product or packaging or that the manufacturer was unable to document that the product was manufactured in a state of control (2). Within the manufacturing environment, poor procedures, practices, and controls during the manufacture of sterile drug products and poorly designed environmental monitoring programs are frequent inspectional observations. Persistent bioburden and biofilm contamination have been implicated in at least one major recall that resulted in a medical shortage of parenteral products (1).

Management of bioburden, primarily biofilms, in pharmaceutical production processes is a major focus of quality programs. Yet, despite the significant resources used in bioburden contamination control efforts, bioburden contamination of manufacturing processes can be a significant cause of compromised product quality and adverse regulatory findings. Reasons for the persistent challenge of bioburden control include production processes that support microbial growth, use of nonsterile source materials, and human interfaces. This challenge is further complicated by the ability of microorganisms to survive and often flourish even in harsh environments (e.g., exposure to chemical sanitizers and disinfectants, high shear, and pressure). The formation of complex, adherent bacterial colonies (“biofilms”) in fluid handling systems is a common adaptive strategy for many microorganisms and presents a significant challenge for their detection and control. Microorganisms growing within biofilms, along with planktonic cells that are present in a bulk phase environment, comprise bioburden in fluid handling systems.

In the latter part of the 20th century, there was a fundamental shift in the understanding of microbial growth in various environments. The commonly held historical perception of bioburden was that it consisted of individual planktonic (free-floating) organisms. The planktonic model has been the basis for most current bioburden management strategies; nearly all of the commercially available bioburden detection systems are based on planktonic cell detection. However, evidence accumulated over the past three decades suggests that biofilms are actually the preferred mode of microbial growth (3-5), with sessile cells sometimes outnumbering planktonic organisms by several orders of magnitude in a given environment. Since the planktonic model does not provide for adequate detection and control of biofilms, there is a need for an increased focus on the development of effective strategies and techniques for the detection and control of biofilms in overall bioburden prevention and control activities.

Microbial and, in particular, biofilm control remains a major challenge for the medical device and pharmaceutical industries. Similarly, in hospitals and other healthcare institutions, a knowledge gap exists in the detection and control of biofilm-related infections (6).

1.1 Scope and Purpose

A comprehensive program of bioburden management includes strategies for preventing and controlling biofilms and is based on current scientific knowledge of microbial growth and adaptation. This technical report presents the current scientific understanding of the causes of, and control strategies for, bioburden in pharmaceutical production systems, with a special emphasis on biofilms in fluid-handling systems. The scope of the report encompasses pharmaceutical and biopharmaceutical manufacturing processes but does not include final aseptic and terminal sterilization fill-finish operations. It is important to educate engineers, scientists and managers about the science of bioburden and biofilms because of the broad and complex challenge of bioburden management.

Specifically, the report provides:

- An overview of the current understanding of the fundamentals of bioburden and biofilm science
- Comprehensive design principles for bioburden prevention and control
- Practical guidance for operational prevention of bioburden and biofilms
- A review of current bioburden detection methodologies and measurement techniques with a focus on biofilms
- Remediation strategies for bioburden and biofilm contamination