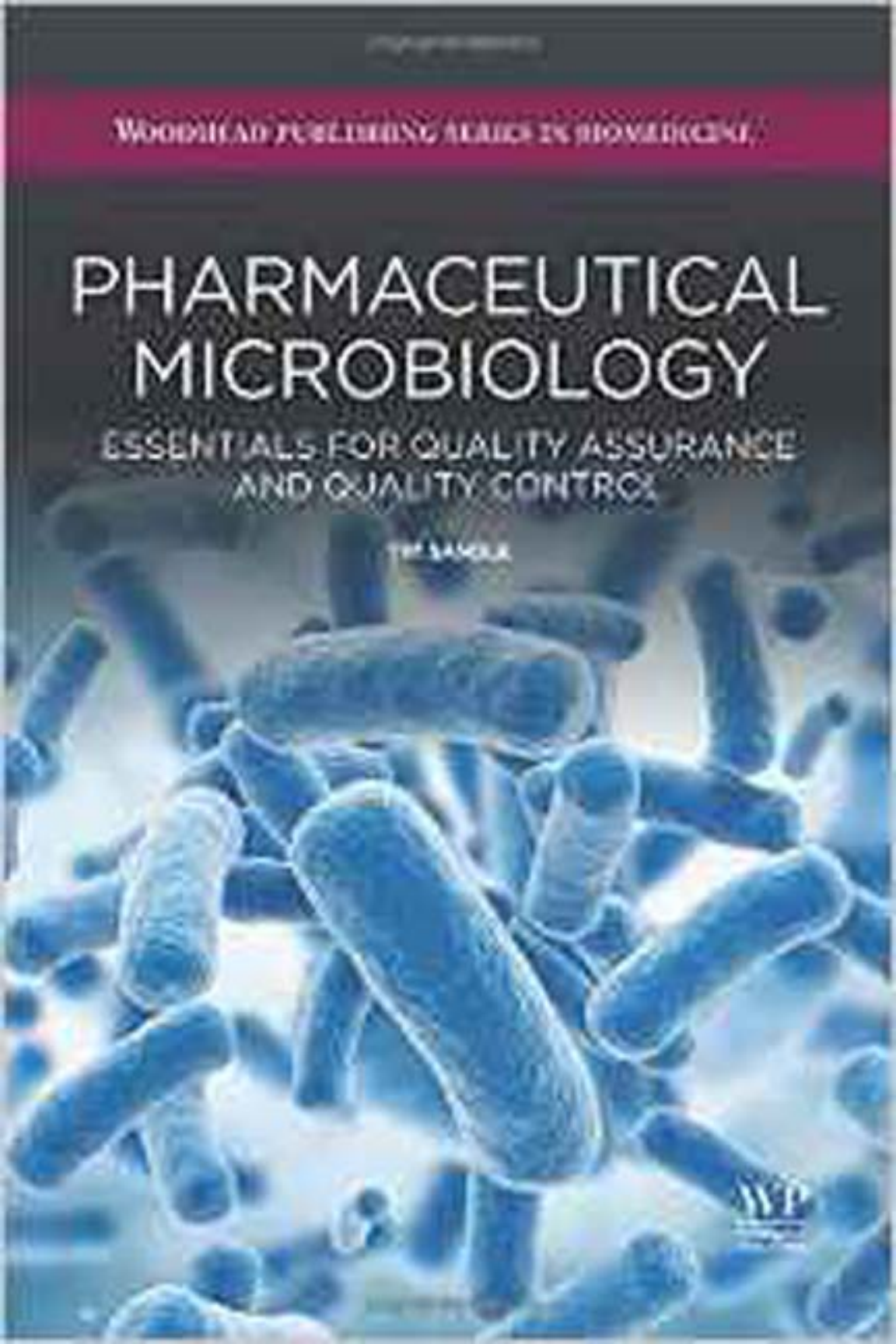
WOODHEAD PUBLISHING SERIES IN BIOMEDICINE

# PHARMACEUTICAL MICROBIOLOGY

## ESSENTIALS FOR QUALITY ASSURANCE AND QUALITY CONTROL

TIM SANDLE



# Pharmaceutical Microbiology

## Related titles

*Sterility, Sterilisation and Sterility Assurance for Pharmaceuticals*
(ISBN 9781907568381)

Woodhead Publishing Series in Biomedicine:
Number 80

# Pharmaceutical Microbiology

## Essentials for Quality Assurance and Quality Control

*Tim Sandle*

AMSTERDAM • BOSTON • CAMBRIDGE • HEIDELBERG
LONDON • NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Woodhead Publishing is an imprint of Elsevier

Woodhead Publishing Limited is an imprint of Elsevier
80 High Street, Sawston, Cambridge, CB22 3HJ, UK
225 Wyman Street, Waltham, MA 02451, USA
Langford Lane, Kidlington, OX5 1GB, UK



## Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

ISBN: 978-0-08-100022-9 (print)
ISBN: 978-0-08-100044-1 (online)

**British Library Cataloguing in Publication Data**
A catalogue record for this book is available from the British Library

**Library of Congress Cataloging-in-Publication Data**
A catalog record for this book is available from the Library of Congress

# Dedication

"This book is dedicated to my wife Jenny, for her invaluable encouragement support."

# Introduction

This book is concerned with pharmaceutical microbiology. Pharmaceutical microbiology is a relatively new discipline, or at least, one defined as a distinct subject matter. A trawl though textbooks pre-dating the mid-1980s reveals very few references to this sub-division of microbiology. Pharmaceutical microbiology can be simplified as being concerned with keeping microorganisms in control within the environment and hence from contaminating the product and harnessing microorganisms to make efficacious pharmaceutical medications [1]. Here pharmacists and microbiologists work synergistically to ensure that drug therapies target opportunistic microorganisms without harming its human host.

In considering the two facets of pharmaceutical microbiology, terms of product protection, pharmaceutical microbiology is concerned with minimizing the numbers of microorganisms within a process environment and excluding microorganisms and microbial by-products (such as bacterial exotoxin) from water and other starting materials. With sterile products, there is an additional requirement: ensuring that the finished pharmaceutical product is sterile. With the utilisation of microorganisms, pharmaceutical microbiology is concerned with the study of anti-infective agents (such as new antibiotics); the use of microorganisms to detect mutagenic and carcinogenic activity in prospective drugs; as well as the biotechnological application of microorganisms in the manufacture of pharmaceutical products, such as insulin.

Pharmaceutical microbiologists operate within both quality assurance and quality control. They are engaged in laboratory testing and environmental monitoring to support these activities; and acting as subject matter experts on topics as diverse as sanitization, autoclave operation, depyrogenation, process validation, selecting cleanroom gowns, specifying raw materials, and so on.

What this book does is to take pharmaceutical microbiology and place it at the heart of the pharmaceutical organization or healthcare institution. Here the book addresses a number of themes, such as drug development, the use of microorganisms in manufacturing, contamination control strategies, laboratory test methods and risk assessment.

There are a few volumes of pharmaceutical microbiology-related books available. These texts tend to focus heavily on the role of microorganisms in biotechnology or the removal or elimination of microorganisms through practices of disinfection and sterilization. Some other texts discuss laboratory test methods in isolation. What this book does is embrace these two themes, expand upon them and introduce a range of other topics that the pharmaceutical microbiologist needs to contend with. Here the author considers that the pharmaceutical microbiologist should only be spending a small amount of time in the laboratory. The establishment and qualification of test methods is important; however, the microbiologist is best served walking the plant, discussing new product development with research and development (R&D), being involved in cleanroom design and air-handling system upgrades, and, perhaps, foremost,

undertaking risk assessments. The types of risk assessment that the microbiologist is required to be involved with are either an assessment of the significance of an above action level result where corrective or preventative actions (CAPAs) are employed or, more commonly, an assessment of the controls and measures in place to ensure that the above action result does not occur in the first place. In essence, being proactive rather than reactive.

This book is aimed at pharmaceutical microbiologists, with the idea of offering a different perspective on longstanding areas of testing (such a bioburden) as well as the new (rapid microbiological methods and risk assessment); at students of microbiology, pharmacy and healthcare subjects; and at personnel working in the field of pharmaceuticals, medical devices, biotechnology and healthcare, who wish to gain an understanding of the discipline or a specific area, such as disinfection or sterilization.

In terms of the structure of this book, Chapter 1 is an introduction to pharmaceutical microbiology, including a basic undertaking of microbial growth [2]. The chapter considers some of the key tests undertaken in pharmaceutical microbiology laboratories and outlines these along the path of pharmaceutical manufacturing (from starting materials to finished products). The chapter also addresses testing in support of products, utilities and the assessment of cleanrooms. Some basic matters in terms of counting plates, sampling, speciation, and risk assessment (including the necessity of a contamination control strategy) are addressed. The chapter provides a stand-alone introduction to the subject matter.

Building on the first chapter, Chapter 2 presents an outline of the pharmaceutical industry with a focus on the role of the microbiologist within it. For those readers unfamiliar with the intimate working of the pharmaceutical industry, the chapter outlines the different aspects and the process of drug development. Pharmaceutical development and manufacture are regulated through good manufacturing practice (GMP). Chapter 3 looks at GMP and compliance and, at the same time, compares and contrasts the approach of international regulators such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Included in the chapter is the importance of key pharmacopeia, which provide the basis of many laboratory tests. To be effective, the pharmaceutical microbiologist needs to understand the compliance arena.

Chapter 4 looks at an oft overlook aspect, and this is with the design, set-up and management of the microbiology laboratory. As well as good design principles, the chapter considers laboratory safety and associated microbiological risks and the qualification of equipment. The chapter, with a nod towards total quality management, introduces the topic of “lean labs” and the issue of harnessing laboratory resources efficiently.

Microbiological culture media is the foundation to many aspects of microbiology [3]. Even with the dawn of rapid microbiological methods, growing microorganisms is essential (and many rapid microbiological methods are, in fact, growth based). Chapter 5 discusses the different types of culture media, variations of the testing regimes designed to release culture media into the laboratory, and the important steps that need to be followed when manufacturing culture media.

Chapter 6 continues with the microbiology laboratory and reviews the principle methods that form part of the pharmaceutical microbiologist’s armory. Considered

here is good laboratory practice, health and safety, aspect technique, microscopy, and culture-dependent methods. From this, the tests considered are total viable aerobic count, tests for specified microorganisms, optical density measurements, the test for sterility, pyrogen testing, endotoxin testing, and water analysis. With each test, aspects relating to method validation are highlighted, together with best practice advice.

The following chapters discuss the more important microbiological methods in more detail. Chapter 7 is concerned with bioburden testing. The chapter presents the main test methods for conducting bioburden, including plate counting (pour plates, spread plates, and membrane filtration) and the most probable number technique. An important discussion is contained within the chapter in relation to the "colony-forming unit" (what it is and what it is not) and the complexities of counting microorganisms in terms of under- and over-estimation. For readers interested in sterile products, a section is included on pre-final filtration bioburden determination. Chapter 8 is about objectionable and specified microorganisms. Although the specified microorganisms listed in the major pharmacopoeia cannot be overlooked, the emphasis in the chapter is upon risk and the importance of the microbiologist understanding the products and processes so that those microorganisms that could damage the product or cause harm to the patient are characterized and tracked. This additionally requires knowledge of the interaction between microorganisms and people, which is addressed through an introduction to the metagenomics of the Human Microbiome Project.

Understanding the types of microorganisms, as use as controls in testing, to confirm organisms used in product development or to identify contamination is a core part of pharmaceutical microbiology [4]. With contamination events and out-of-limits events, failure to characterize invariably leads to a failure to conduct an investigation soundly. Chapter 9 is about microbial identification and the chapter presents an array of phenotypic and genotypic methods. Given that choice between competing methods can often be bewildering, the chapter offers a matrix that can be adopted for selection and qualification.

Water is an important concern for microbiologists and the use of water is an inescapable feature of pharmaceutical production. Water, when produced and controlled properly, plays an effective role. However, poor design and control can lead to severe microbiological issues, not least because water acts as both a growth source and a vector for microbes. Chapter 10 looks at supplied potable water and the purification process that generates purified water and water for injections. As well as good design principles, the chapter also discuss the types of microorganisms found and requirements of testing for viable counts, bacterial endotoxin and total organic carbon.

Closely related to water is bacterial endotoxin, which is the major source of pyrogens within the pharmaceutical organization. Chapter 11 looks at the nature and sources of endotoxin (and Gram-negative bacteria) and the nature and risks of pyrogenicity. In addition, there is an in-depth review of the primary test for endotoxin: *Limulus* amebocyte lysate (LAL) method.

Chapter 12 places microbiology at the core of many production processes and examines sterility and sterility assurance. The microbiologist needs a core understanding of sterility assurance principles, whether this is for sterile products or medical devices, or for the consumables used within cleanrooms and laboratories [5]. The chapter examines

the theory of sterilization and some of the different methods that can be deployed to achieve it. The theme of sterility is extended to Chapter 13, which considers biological indicators (preparations of microbial spores) used to qualify sterilization (such as moist heat, dry heat, ethylene oxide, and hydrogen peroxide). The chapter discusses concepts such as *D*-value, population and identification, with a focus on biological indicator resistance.

Chapter 14 examines antimicrobials. An antimicrobial is any substance of natural, semi-synthetic or synthetic origin that kills or inhibits the growth of microorganisms but causes little or no damage to the host. In undertaking this, the chapter considers two important aspects of pharmaceutical product efficacy. The first is the assessment of antibiotics to determine if the antibiotic is effective against the target microorganism. The second is the assessment of pharmaceutical products that contain preservatives, where testing is undertaken in order to assess whether the preservative can suppress the growth of any contaminating microorganisms.

Cleaning and disinfection are of great importance to process environments. Without efficient cleaning (removal of soil) then disinfectants will not work effectively, and without a robust disinfection programme, then microorganisms will be present, either numerically or in terms of problematic species, at levels that present an environmental challenge to the product [6]. Chapter 15 presents an overview of detergents and disinfectants, and discusses issues of qualification and best practice advice for the use of such agents within a GMP environment.

Pharmaceutical processing needs to take place within a controlled environment and, in most cases, there is the requirement for a classified cleanroom. Chapter 16 reviews the design requirements for cleanrooms (air handling systems and the role of air in removing contamination). The chapter also discusses clean air devices, such as isolators and safety cabinets. The chapter devotes space to environmental control and environmental monitoring, and describes the fundamentals for designing an environmental monitoring programme as part of a bio-contamination control strategy.

Many of the laboratory methods used in pharmaceutical microbiology have remained unchanged for decades (some, such as classic counting methods being largely unchanged since the nineteenth century). The twenty-first century has seen the advent of rapid microbiological methods. These methods are designed to provide a faster time-to-result and/or a more accurate assessment of microbial numbers. Chapter 17 assesses the different types and technologies of rapid methods available, and discusses how the microbiologist can be best placed to select from different (and at times competing) technologies.

The main contribution that the pharmaceutical microbiologist can make to ensuring the safety of medicines is through risk assessment. Chapter 18 presents an introduction to risk assessment and its alignment with current GMP. The chapter then considers some risk assessment tools and techniques before moving on to outline some of the main sources of contamination within the facility. The chapter then presents some examples of risk assessment tools based on surface and air contamination. These examples show that risks can be quantified, compared and contrasted.

Chapter 19 describes another important area where pharmaceutical microbiologists should be at the forefront: validation of pharmaceutical equipment. Most items of

equipment are designed to be self-sanitizing or self-sterilizing, or, alternatively, this has to be carried out manually. The pharmaceutical microbiologist must be closely involved in this process. The chapter describes the essential of validation, running through installation, operation and performance qualification. The importance of contamination control is illustrated through the example of cleaning validation. In addition to validation, the microbiologist should understand the batch manufacturing process and its constituent steps. The chapter describes this process fully and pinpoints where microbiological input is required.

Microbiological testing is of little value if the data are not analyzed. Chapter 20 is about the analysis and interpretation of microbiological data. This task is not as straightforward as with other biological data because microbial data rarely conform to standardized normal distribution. The marketed skewness of microbial data either requires the data to be transformed or for alternatives methods to be used. The handling of data is discussed with a view to producing control charts. A second area that the microbiologist needs to undertake frequently is the setting of alerts and action levels. The chapter presents ways to set such limits.

An efficient microbiology laboratory must conform to accepted standards, whether this be to GMP or International Organization for Standards (ISO) or to a national accredited body. The best means to assess these standards is through regular audit. Chapter 21 is about auditing the microbiology laboratory. The chapter will be of interest to readers who need to undertake auditing and/or work in laboratories that are subject to audit. The chapter discusses the audit process and the main areas that the auditor should focus on.

The final chapter of the book, Chapter 22, acts as a conclusion. In completing this task, the chapter thematically draws together the key points made throughout the text. The title of the chapter is "Microbial Challenges to the Pharmaceutical Industry." The chapter looks at microbial risks to pharmaceuticals, including points where contamination can arise and how risk assessment is the most effective tool to avoid such contaminating events. The chapter also considers the microbial risks to process environments. In running through these sources, the chapter ties together the main issues of contamination concern.

In outlining these chapters, it can be seen that the book seeks to place pharmaceutical microbiology at the forefront of pharmaceuticals and healthcare. Medicines may be efficacious in terms of their therapeutic value but this is of little value if the medicine is contaminated, for the therapeutic value may be lost or the medicine may be dangerous to the patient. The book also seeks to unshackle the microbiologist from the bench, equip him or her with risk assessment tools and process understanding, and position the microbiologist at the heart of the drug development and manufacturing process.

## References

[1] Sandle T, Saghee MR. The essentials of pharmaceutical microbiology. In: Saghee MR, Sandle T, Tidswell EC, editors. Microbiology and sterility assurance in pharmaceuticals and medical devices. New Delhi: Business Horizons; 2011. p. 1–30.

[2] Srivastava S, Srivastava PS. Understanding bacteria. Dordrecht: Kluwer Academic Press; 2003, p. 28.
[3] Sandle T. The media kitchen: preparation and testing of microbiological culture media. In: Sutton S, editor. Laboratory design: establishing the facility and management structure. Bethesda, MD: Parenteral Drug Association; 2010. p. 269–93, ISBN: 1-933722-46-0.
[4] Jimenez L. Microorganisms in the environment and their relevance to pharmaceutical processes. In: Jimmenez L, editor. Microbiological contamination control in the pharmaceutical industry. New York: Marcel-Dekker Inc.; 2004. p. 3–7.
[5] Sandle T. Practical approaches to sterility testing. J Validation Technol 2004;10(2):131–41.
[6] Sandle T. Cleaning and disinfection. In: Sandle T, editor. The CDC handbook: a guide to cleaning and disinfecting cleanrooms. Surrey, UK: Grosvenor House Publishing; 2012. p. 1–31.