# **Nicrobiology LABORATORY THEORY** & APPLICATION



Michael J. Leboffe & Burton E. Pierce SECOND EDITION





Michael J. Leboffe

San Diego City College

Burton E. Pierce



#### **Book Team**

Editorial Assistant: Rayna Bailey Production Manager: Joanne Saliger Production Assistant: Will Kelley

Publisher: Douglas N. Morton Biology Editor: David Ferguson Cover Design: Bob Schram, Bookends, Inc.

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ISBN 13: 978-0-89582-947-4

Library of Congress Control Number: 2011942014

10 9 8 7 6 5 4 3

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Printed in the United States of America



elcome to the second edition of Microbiology Laboratory Theory and Application, Brief Edition! The response to the first edition was incredibly positive and we are grateful to the adopters for its reception and their support. Many made useful suggestions for improvement, which have been incorporated herein.

We have made a couple of significant changes and many other more subtle changes. Probably the most noticeable change is the use of professionally rendered artwork throughout the manual. While most of these convey the same information as the originals upon which they were based, they are many times more attractive. Beyond replacing all the artwork, we have replaced or added more than 50 new photographs.

The second, more substantive change is reduction in the use of BSL-2 organisms. Where possible, BSL-2 organisms are replaced with equivalent BSL-1 organisms. However, in some instances, a particular exercise required the use of a BSL-2 organism to work, so it is retained with appropriate caution advised. An example of this is Staphylococcus aureus in the coagulase test. In a few places, you will see the use of a "surrogate" organism. An example of this is shown in Exercise 4-6 (Hektoen Enteric Agar). The strain of Providencia stuartii (BSL-1) produced the same result on the medium as Shigella flexneri (BSL-2) and was, therefore, the obvious alternative. However, because one of the purposes of the medium is to isolate Shigella species, we decided to retain S. flexneri in the recommended organism list with P. stuartii, in parentheses, as a surrogate. Other changes by section are outlined below.

#### Introduction

Minor changes were made to update the information on BSL categories and strengthen the emphasis on safety. A short treatment of chemical hazards and their standard labeling was added.

#### **Section 1**

A new, simple lab comparing the effectiveness of several hand-cleansing agents was added (Exercise 1-1). More importantly, Exercise 1-4 (Common Aseptic Transfers) was rewritten and, we hope, streamlined. Procedural diagrams were also added as was extra emphasis on handling microbes in such a way as to reduce potentially dangerous aerosols. Several new and replacement photos were added. And, we followed the advice of our reviewers to emphasize labeling media before inoculation rather than afterward.

## Preface

#### Section 2

Several new photos were added to improve this section. Exercise 2-13 (Chemical Germicides) was moved to the end of the section and was rewritten to include a more comprehensive procedure using BSL-1 organisms.

#### Section 3

Exercise 3-1 (Introduction to the Light Microscope) now includes two activities to supplement the theory and rules of microscope usage. These are the letter "e" slide to familiarize students with the inverted image and the colored threads slide to promote practice at fine focusing. Exercise 3-3 (Eukaryotic Microbes) was totally reorganized to reflect current (but still provisional) classification of these organisms by "Eukaryotic Supergroups." Added to Exercise 3-6 (Gram Stain) is artwork illustrating the differences between Gram-positive and Gram-negative walls. The Acid-Fast Stain (Exercise 3-7) now includes the Ziehl-Neelsen protocol in addition to the Kinyoun method. Throughout, some older photomicrographs were replaced with new ones.

#### Section 4

These exercises were reordered slightly to reflect a more reasonable approach to the material. The photo in Exercise 4-1 (Phenylethyl Alcohol Agar) was replaced with a better one and the Bile Esculin Test was moved to Section 5.

#### Section 5

Several exercises, previously in Section 4 and Section 9, were moved to Section 5. Now included in this section are Bile Esculin Test (Exercise 5-10), PYR Test (Exercise 5-17), Bacitracin, Novobiocin, Optochin Tests (Exercise 5-20), and CAMP Test (Exercise 5-22).

#### Section 6

New artwork and several new photos and/or photomicrographs improve the look of this section. And on the advice of our reviewers to catch up with the 21st century, we have written all of the dilution schemes in this section for digital pipettes.

#### **Section 7**

New antibiotics were added to the Kirby-Bauer Test (Exercise 7-2) as were a couple of new photos. The *Morbidity and Mortality Weekly Report* (Exercise 7-3) was updated and includes a photo of the CDC. The Epidemic Simulation (Exercise 7-4) was rewritten to place even greater emphasis on the techniques of safely executing the transfers without aerosol or droplet production.

#### **Section 8**

Two exercises were removed: precipitin ring test and the dot blot. The latter was replaced with the Bio-Rad ELISA kit (Exercise 8-6).

#### **Section 9**

Exercises 9-1, 9-2, and 9-3 (Identification of Unknown) were rewritten with new or revised identification flowcharts, necessitated by changing organism inventories at standard biological supply houses. An attempt was made to reduce the number of media required for each identification to provide more options for tests, and to reduce the number of BSL-2 organisms used.

#### **Appendices**

These still provide useful, but supplemental, materials for those that wish to use them. Appendix A (Biochemical Pathways) had a couple of errors removed and some newer information about ATP yields added. Appendix B (Miscellaneous Transfer Methods) was revised to follow the new style used in Exercise 1-3, as were Appendices C and D (involving pipetting). The alternative procedures appendix (formerly Appendix E) was discontinued. All of the alternative dilution schemes using digital pipettors were fully incorporated in Section 6 procedures.

#### Acknowledgments

First, we want to thank everyone who has adopted our microbiology titles over the last 16 years. It all started with A Photographic Atlas for the Microbiology Laboratory in 1995 (now in its 4th edition). Over the years, it has spawned three other titles: Exercises for the Microbiology Laboratory (1996, also in its 4th edition); Microbiology Laboratory Theory and Application (2002, now in its 3rd edition); and Microbiology Laboratory Theory and Application, Brief (2007, with the 2nd edition resting securely in your hands). Clearly, we have benefitted from suggestions for improvement from innumerable people over the last 16 years and we haven't forgotten you.

We want to thank our colleagues and friends at San Diego City College for their continued support. In alphabetical order, these include Donna DiPaolo, Anita Hettena, Roya Lahijani, David Singer, Minou Spradley, Laura Steininger, and Muu Vu. We would particularly like to thank Debra Reed for her involvement and assistance in running many necessary tests and for her work finding safer alternatives to many of the BSL-2 organisms used in the first edition. We also are grateful to San Diego City College for making lab facilities available through the Civic Center Program. It would be next to impossible to write a laboratory manual without a lab to work in! Lastly, thanks to the Biology 205 students over the years (and in particular the Fall 2011 MW and TTh morning sections) who have performed the labs and have pointed out rough spots that were not apparent to us. While we have been the grateful beneficiaries of occasional suggestions from adopters, we especially want to recognize the efforts of Janice Smith, Tarrant County College, Fort Worth, Texas; Johana Melendez, Hillsborough Community College, Tampa Bay, Florida; and Diane Doidge, Grand View University, Des Moines, Iowa, for reviewing the first edition and for their helpful suggestions. Reviewing a complex work of this size is not an easy task and we are indebted to them for supplying fresh eyes to scrutinize its contents.

As always, we are indebted to the people at Morton Publishing. It is hard to imagine a better group of people for authors to work with. Thanks to Doug Morton (President) for his vision of producing high quality, yet affordable, texts for students. Thanks also go to Chrissy Morton DeMier (Business Manager), David Ferguson (Biology/Acquisitions Editor), Carter Fenton (Sales and Marketing Manager), Joanne Saliger (Production Manager), Will Kelley (Production Assistant), and Rayna Bailey (Editorial Assistant) for playing their parts in putting their authors in a position to be successful. Each person listed plays a crucial role in the publication of our works, but special recognition is owed to Joanne Saliger, because her efforts in designing the layout are seen by the readers. The quality of a book is mostly in its content, but extraordinary design surely makes the book more palatable. Thanks also go to Bob Schram of Bookends, Inc. for the cover design. Finally, we thank the talented people at Imagineering Art in Toronto, Ontario, Canada, for their artistic renderings. Their inclusion is probably the most noticeable change in this edition.



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