Preface

I get the best mail.

I get letters (o.k., emails and tweets) from my students, both past and present, about situations they encounter that remind them of microbiology. An infection that required a change in antibiotic treatment, mold-encrusted strawberries, even—and I promise this is true—a smell coming from a storm sewer that a student recognized because it smelled just like her unknown bacterial culture. The point is that the typical microbiology course is often thought of as arcane but is more relevant to our lives than any other class an undergraduate is likely to take. Argue with me? Fine, but remember that the cup of coffee in your hand was made with chemically disinfected water and has pasteurized milk in it. For microbiologists, and those who already intend to enter the field, this battle of relevance has already been won, but for the vast majority of students who are taking microbiology as a prelude to nursing, dental hygiene, or another allied health profession, the science only seems to come alive when linked to everyday experiences. Usually we, as microbiologists, recognize the story in the paper or the blurb on the radio as a living example of the impact of microbiology on our lives and try to share that with our students. Unfortunately, news stories do not always adhere to our syllabi, and many of these wonderful teaching moments go unexploited.

For Whom Is This Lab Manual Written?

Written for students entering the allied health fields, *Laboratory Applications in Microbiology: A Case Study Approach,* is designed to use real-life examples, or case studies, as the basis for exercises in the laboratory. Over the past few years, the number of lecture texts utilizing case studies has grown rapidly, and for good reason—case studies work! This book is the only lab manual focusing on this means of instruction, an approach particularly applicable to the microbiology laboratory. All the microbiological theory in the world means little if students cannot understand the importance of a Gram stain, antibiogram, or other laboratory procedure.

What Sets This Lab Manual Apart?

This book was created to make the microbiology lab a more valuable experience by reconnecting the **what** and **how** of microbiology with the sometimes forgotten **why.** Although Latin names, complex media, and complicated assays will always be a part of the curriculum, the context of each exercise has been expanded so that the reason for completing a specific task will be clear from the outset. Several features of the book are used to accomplish this goal and serve to distinguish it from other microbiology lab manuals.

Case Studies

The first 39 exercises include actual cases taken from the scientific literature. The techniques, media, and observational tools introduced in each exercise help students to solve the issues presented in the case, driving home the relevance of microbiology and honing critical thinking skills. Evidence has shown that the use of case studies boosts learning, develops critical thinking skills, increases retention of students in the classroom, and even reduces the incidence of academic dishonesty. Simply put, students learn more, learn faster, and retain more with case studies than with traditional instruction methods. Although this seems obvious to those of us who cannot wait to share the day's news story with our class, the results are backed up by empirical evidence. In one study focused on instructors who use cases, 97 percent reported that students who were taught with cases learned new ways to think about an issue; 95 percent reported that students took a more active part in the learning process; and 92 percent reported that students were more engaged in classes, truly remarkable numbers.

Photographic Atlas

The photo atlas, located between exercises 39 and 40, contains more than 300 color photos that **show** what a capsule stain should look like, exactly how large a red blood cell is, and how to tell the eggs of a pinworm from those of a roundworm. Because that is what microbiologists—and microbiology students—do. What's not there is a long (or long-winded) discussion of the biology of a schistosome or even a full explanation of why a bacterial cell may be Gram positive as opposed to Gram negative. These discussions are left for the lab manual and textbook.

This atlas is organized from the point of view of a scientist trying to identify a specimen in the laboratory. By illustrating the answers to very basic questions—"What does a mold look like?" "Is my endospore stain positive?" "Is that

an oocyst?"—students can evaluate their work with the eye of someone who has seen these things before.

The photos of a single organism are often spread throughout the atlas. The reason for this again goes back to the manner in which you would examine a specimen in the lab. A student wouldn't, and in fact couldn't, examine a bacterial specimen both macroscopically and microscopically at the same time, so those images won't be found next to one another. What is found in every magnified image, however, is an indication of the magnification used to acquire it. In most cases, knowing what a specimen looks like when viewed at a magnification of 1000× is generally more useful than knowing that it is 7 μm in length.

The atlas is divided into eight major sections as follows:

- Staining techniques
- Cultural and biochemical tests
- Bacterial colonial morphology
- Bacterial microscopic morphology
- Fungi (both macroscopic and microscopic images)
- Protists
- Helminths
- Hematology and serology

Within each section, of course, microbes are further organized. For example, protozoans are classified by motility: amoeba, flagellates, ciliates, and apicomplexans. When a student comes across a flagellated protozoan in their studies, they should know right where to go.

Flip Your Classroom!

It's always good when a great idea gets a name. Hardly a new concept, "flipping" refers to students accessing basic information before coming to class, leaving more time during class for problem solving, collaborative learning, and the development of higher order critical thinking skills. From the beginning, *Laboratory Applications in Microbiology* has been designed for the flipped classroom. Student learning outcomes, extensive laboratory introductions, and pre-lab questions all combine to let students begin their learning at home, maximizing the scarce time available in the lab, and freeing teachers to spend more time . . . teaching.

Changes to the Third Edition

When Apple released its latest iPhone, it rolled out an advertising campaign focused on the fact that the new phone was both bigger (in terms of screen size) and smaller (at least thinner) at the same time. The subtext, of course, was that the phone had more of what people wanted, less of what they didn't, and was easier to use, all at the same time.

In that same spirit, the third edition of *Laboratory Applications in Microbiology* has incorporated a number of new features designed to keep the manual contemporary, make it easier to use, and improve the experience of both students and instructors. These changes include:

- New feature. "There's More to the Story . . . " serves as a jumping off point for students who want to go the extra mile. Broadly written, these ten addendums ask students to take the exercise they've just completed to the next step. After Exercise 17 (Lethal Effects of Ultraviolet Light) for example, students are encouraged to study the effects of environmental UV radiation on bacterial populations. Subsequent to an exercise on algae, students have a chance to receive training and become volunteer researchers for the Phytoplankton Monitoring Network. Following an exercise on milk spoilage (MBRT, ex 30), students can make—and study—their own fermented food. Whether used as extra credit, individual exercises, or even independent study projects, There's More to the Story . . . requires students to do research, generate a protocol, and prove or disprove a hypothesis, in other words, act like the scientists we are training them to be.
- New organization. The case study for each exercise has been consolidated and moved to the end of the exercise. Exercises have also been reformatted to speed things along and make the case study optional. The laboratory procedure now immediately follows the introduction, allowing students and instructors to jump right in to lab work. Questions too have been rearranged and reformatted so that lower-order questions (knowledge, comprehension) appear first, with higher-level questions (analysis) following the lab exercise. The case study follows the lab and is followed in turn by several highest-order questions (synthesis and evaluation) based specifically on the events detailed in the study.
- New icon. Throughout the manual you will see a camera icon like this directing you to a specific page in the photo atlas. On that page you will find photos of organisms, cultural characteristics, biochemical or physiological results that will prove helpful.

- New case studies. Some interesting things have happened in the last few years, and you'll find a dozen new case studies this time around. Exercise 1 on laboratory safety for instance now follows a nationwide outbreak of *Salmonella* that began in a microbiology teaching lab. It literally cannot get any more relevant. Additionally, cases throughout the manual have been updated to include any new information.
- New student learning outcomes (SLOs) have been added to each exercise. Found as part of the case study exercises in the second edition, expanding SLOs to the

entire manual allows concrete evaluation of the skills and theory a student should master as they complete each exercise. The SLOs also allow instructors to easily track the skills they teach and correlate these to department and campus objectives.

- New photographs. Over three dozen new photographs can be found throughout the manual. Chosen because they display new techniques, alternative methods, or simply because each was better than the photograph it replaced, every new photo was chosen with only one thought in mind, how to make microbiology more understandable to the student.

A more comprehensive list of changes to the third edition includes the following:

Exercise 1: Laboratory Safety
- A new case study focusing on a nationwide *Salmonella* epidemic that had its start in a microbiology teaching laboratory.
- Updates to a UCLA lab fire, including the legal consequences for faculty.

Exercise 3: Identification and Classification of Algae
- New case study on toxic algae.
- There's More to the Story . . . Algae monitoring as part of the Phytoplankton Monitoring Network.

Exercise 4: Medically Important Protozoa
- New case study on *Naegleria fowleri* infections connected to sinus rinsing.

Exercise 5: Identification and Classification of Fungi
- New case study on *Histoplasma* infections connected to a summer day camp.
- New information concerning fungal pathogenesis.

Exercise 6: Ubiquity of Microorganisms
- Three new case studies on hospital design and infection control.

Exercise 7: Aseptic and Pure Culture Techniques
- Pure culture and aseptic techniques have been combined into a single exercise.

Exercises 8–11: All staining exercises have been supplemented by including information previously found only in the laboratory techniques section of the manual.

Exercise 10: Endospore Staining
- A single exercise is completely devoted to endospore staining.
- There's More to the Story . . . Isolation and identification of endospore-forming bacteria.

Exercise 11: Acid-Fast Staining
- A single exercise is completely devoted to acid-fast staining.
- New case study on *Mycobacterium chelonae* infections connected to tattoos.

Exercise 12: Viable Plate Count
- New case study concerning *E. coli* O157:H7 infections associated with raw milk.
- Additional information on the importance of coliforms.
- More background on viable plate counts along with tips to improve the success and reproducibility of the exercise.

Exercise 16: Effect of Osmotic Pressure on Bacterial Growth
- There's More to the Story . . . Isolation of Halotolerant Microbes.

Exercise 17: Effects of Ultraviolet Light
- There's More to the Story . . . Environmental effects of ultraviolet light.

Exercise 18: Evaluation of Disinfectants
- New case study concerning skin infections traced to wrestling equipment.
- There's More to the Story . . . Disk Diffusion Testing of Disinfectants.

Exercise 19: Hand Scrubbing
- There's More to the Story . . . Analysis of Skin Microbiota.

Exercise 20: Antimicrobic Susceptibility Testing
- There's More to the Story . . . Isolation of Antibiotic-Producing Bacteria.

Exercise 24: Bacterial Transformation
- New case study, Hospital Outbreak of Carbepenem-Resistant Enterobacteriaceae.

Exercise 26: DNA Extraction from Bacterial Cells
- New case study, Ikea Pulls Meatballs from Store Shelves after Horsemeat DNA Detected.

Exercise 29: Membrane Filtration
- There's More to the Story . . . Identification of Bacteria in Water.

Exercise 30: Methylene Blue Reductase Test
- There's More to the Story . . . Production and Study of Yogurt.

Exercise 33: Isolation and Identification of Streptococci
- New case study, *Streptococcus pneumoniae* Outbreak in Nursing Facility.
- There's More to the Story . . . Tracking Hand Hygiene

Exercise 34: Differentiation of Enterobacteriaceae
- *E. coli* O157:H7 outbreak tied to a Petting Zoo.

Progression of Exercises Promotes Active Learning

Material in each of the first 39 exercises has been carefully organized so that students develop a solid intellectual base, beginning with a particular technique, mastering it, and then

applying this new knowledge to a case study. Immediately following the introductory material, pre-lab questions help students to focus on the important aspects of a technique, developing a framework for what they will need to do **prior to the lab,** many of which require two or three periods. Between the multiday labs, questions are posed to ensure that the students understand **what** they have just done, the **results** they should expect, and the **significance** of those results. Post-lab questions require applying the knowledge gained from the exercise to answer more thought-provoking questions about the techniques they have just studied. Each of the first 39 exercises concludes with a case study, a real-life situation in which the technique just mastered plays a starring role. Case study questions, generally higher-order thought questions, challenge students to apply the information they've learned to other situations. In a quarter of the exercises, open-ended topics for study are featured (There's More to the Story . . .) that allow students to move beyond the everyday and become true researchers.

While the first 39 exercises focus on case studies, the **why** of microbiology, the **how** of the subject has not been forgotten. The final 50 exercises serve as a thorough compendium of common microbiological methods. These exercises are presented in such a way that students will develop critical thinking skills simply by deciding on a particular course of action. All similar techniques, such as selective and differential media or biochemical tests, are grouped together, and each exercise begins with student learning objectives and a brief overview. By reviewing the overview, a student may select an appropriate test, media, or staining technique from the many available, ensuring that they have decided not only what information they need, but how to go about getting it. Written to clearly guide students while also pointing out the importance of a particular technique, this portion of the manual provides detailed, well-illustrated procedures that stand by themselves or can be used in conjunction with the case studies in the front of the book. This is particularly helpful when undertaking unknowns, as each student's unknown culture will require a unique set of procedures for complete identification. A data sheet in the appendix provides a single location for students to record their test results, reinforcing the importance of record keeping in the laboratory.

Extensive Flowcharts for Bacterial Identification

Exercise 39 introduces the concept of bacterial identification, using a case study recounting the recognition of *Legionella pneumophila* as the causative agent of Legionnaires' disease. Within this exercise, 31 flowcharts are used to help identify bacterial unknowns commonly seen in the microbiology laboratory, a far more extensive collection than the one or two found in most manuals. This exercise also serves as an introduction to the techniques section of the manual, allowing students to quickly decide which diagnostic techniques are applicable to their particular unknown culture.

A Self-Contained Resource for the Microbiology Laboratory

In the workplace, allied health professionals are expected to evaluate a situation and find a solution using whatever resources are available to them. This book serves as a self-contained resource, with everything a student needs to solve a problem in the microbiology laboratory. A **glossary** provides definitions of all microbiological terms used in the book, a rarity in the field. **Appendices** contain the formula of every medium and reagent used, in addition to **tutorials** covering universal techniques such as the use of pipettes and spectrophotometers as well as the preparation of media. Each exercise also includes a **link to applicable websites,** such as the CDC homepage for each pathogenic microorganism encountered. In short, this book will help students develop the ability to solve problems.

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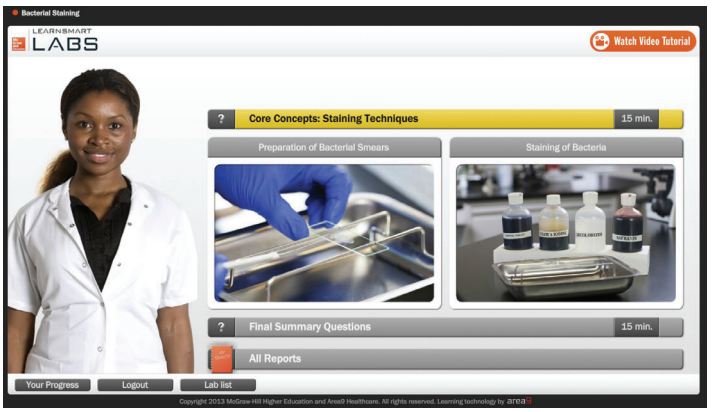

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Student Resource

Annual Editions: Microbiology 10/11 (0-07-738608-6). *Annual Editions* is a series of volumes, each designed to provide convenient, inexpensive access to a wide range of current articles from some of the most respected magazines, newspapers, and journals published today. *Annual Editions* are updated on a regular basis through a continuous monitoring of over 300 periodical sources. The articles selected are authored by prominent scholars, researchers, and commentators writing for a general audience. The *Annual Editions* volumes have a number of common organizational features designed to make them particularly useful in the classroom: a general introduction; an annotated table of contents; a topic guide; an annotated listing of selected World Wide Web sites; and a brief overview for each section. Visit www.mhhe.com/cls for more details.

Acknowledgments

I love all the shows on television that have a single heroic figure surviving in the wilderness. He catches food, builds his boat, survives storms, and signals for rescue, all by himself. Uh, right. Except for twelve cameramen, sound engineers, lighting directors, grips, union representatives . . . you get the idea. Well, when I see my name on the front of this book, I feel much the same way. Without the help and support of a whole bunch of people, I'd still be lost in the proverbial wilderness.

The first thank you, as always, goes to my students, who are the ultimate arbiters of whether anything I say or do is good or bad. I know you didn't sign up to be test subjects for every idea that pops into my head, but there is no way I could do this without your good-natured feedback. Please know that you have helped create a better book. In the lab at Pasadena City College a great number of people have supplied ideas, critiques, and criticisms that have helped shape this book. Special thanks to Jessica Igoe, John Stantzos, and Ray Burke. Of course, nothing happens in the lab without the support of Mary Timmer, laboratory technician of the gods. One colleague at Pasadena City College must be singled out for special recognition. Sonya Valentine read this book from cover to cover and made several hundred suggestions. From simple grammatical mistakes to complex ideas of how an exercise could be better presented, Sonya brought her years of experience as a teacher and microbiologist to bear on this project, and the result is a better book. Thank you Sonya.

Without the people at McGraw-Hill, you would right now be holding my stained, crumpled notes in your hands. Special thanks to product developer Darlene Schueller, who is one of the hardest working people I know (keeping me in line is hard work, ask anyone), and brand manager Amy Reed, who gets my jokes (the highest compliment I can pay a person). Further thanks to project manager Lisa Bruflodt (again, managing me is hard work . . .) and photo research coordinator LouAnn Wilson. All of the people I've had a chance to work with at McGraw-Hill are talented professionals and I count myself lucky to work with them.

Speaking of counting myself lucky. My wife and kids put up with a lot, and each week that they decide not to put me out with the trash I feel blessed. They take it in stride when my office looks like a site in need of FEMA assistance and are kind enough not to rearrange things, disrupting the experiment in chaos theory that is my desk. They know when to offer encouragement and when to offer cheesesteak. Josh, Noah, and Safura, you three mean the world to me, and without your support, I couldn't have done this. I love you all very much.

Reviewers

As always, a talented team of microbiologists had my back. The information they provided about content, procedures, what I got right, and what I could improve upon made this a better book than I could ever have written on my own. My deepest thanks to each of them.

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To the Student

As an introductory student in microbiology, you may find that the reasons behind a particular exercise appear overly complex. Such is the nature of science, but the reasons should, at the very least, be apparent. The first step in closing the chasm between the scientific and the everyday is to understand, always, how each step relates to the overall objective. It is just as important to understand **why** you are doing something as it is to understand **what** it is you are doing. If you can master both the **why** and the **what,** then your success in microbiology will be assured.

This book was written with you in mind, with each feature designed to support something else. Put another way, the introductory material helps to explain the case study, the photos and diagrams are used to clarify procedures, the glossary contains definitions of microbiological terms, and websites are provided if you would like further information on a topic. When you are using this book, please, **use** this book. If the meaning of a sentence is unclear, look to the accompanying figure; if a word is a mystery, use the glossary; if space is provided for a detailed drawing, give it your best shot—it will all be important soon. A well-used book becomes weathered as knowledge moves from the book to the reader, and a lab book is no different in this regard. Dog-eared pages, drawings, notes, and circled definitions are all part of learning, and the physical process of making the book yours parallels the intellectual process of making the information yours. This is as true with microbiology as it is with any other interest, job, or hobby. Take the steps to own the book, and you'll own the information within. Good luck. Work hard and have fun.