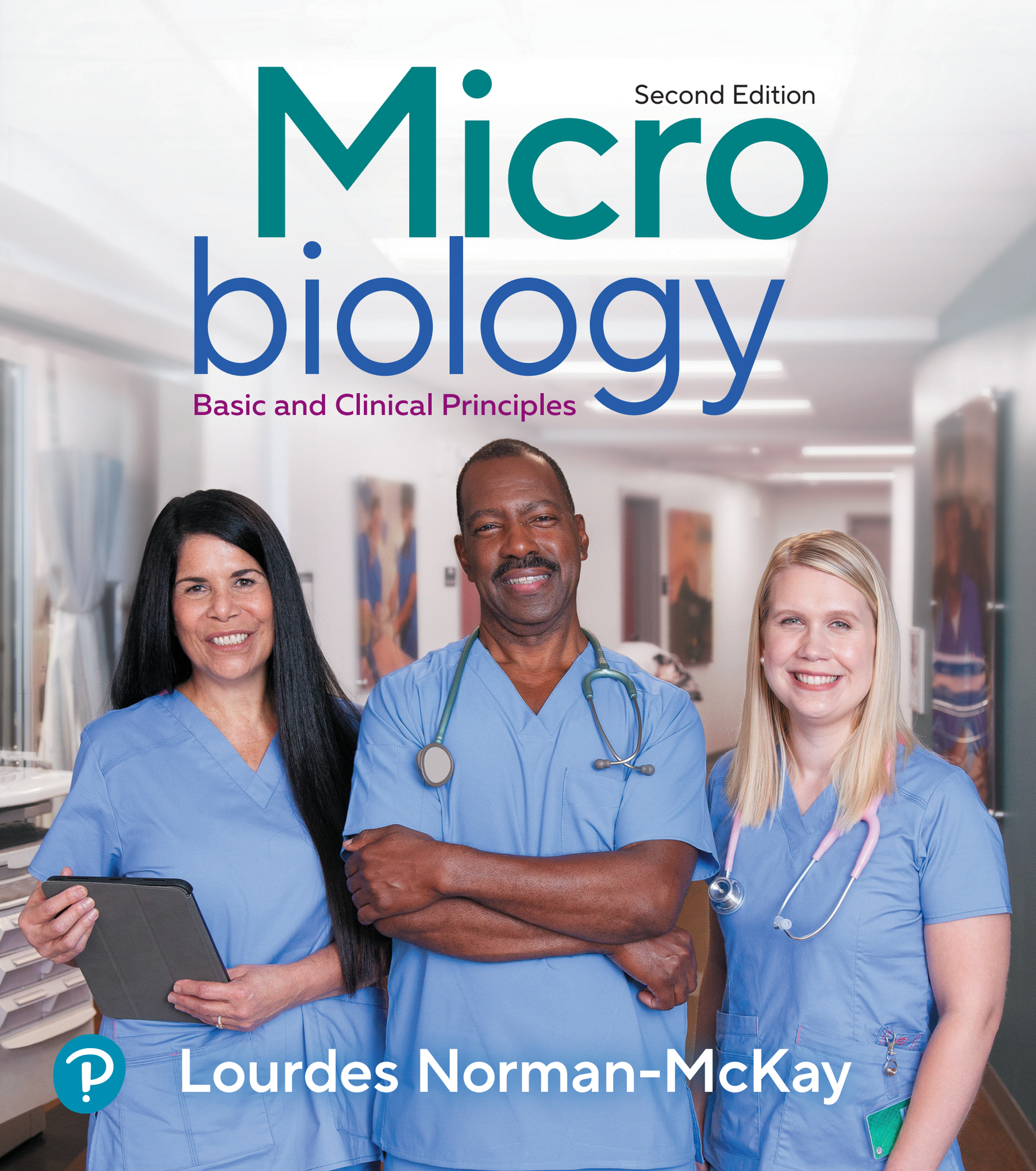
Second Edition

Microbiology

Basic and Clinical Principles



Lourdes Norman-McKay

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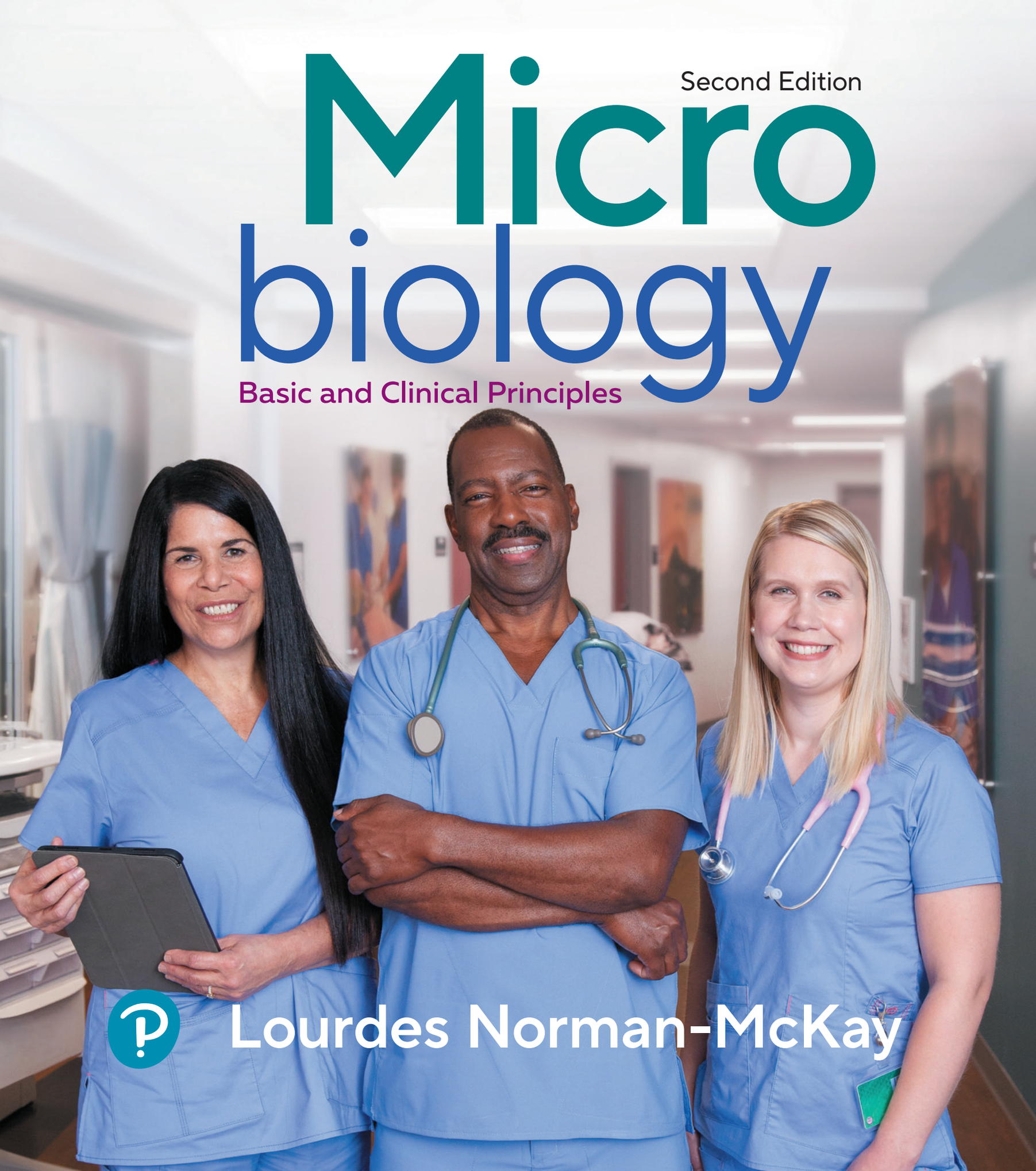

Lourdes Norman-McKay

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About the Author

DR. LOURDES NORMAN-MCKAY is a professor at Florida State College Jacksonville, where her peers and students have recognized her with the Outstanding Faculty Award. She earned her B.S. in microbiology and cell science from the University of Florida and her Ph.D. in biochemistry and molecular biology from the Pennsylvania State University College of Medicine. Her postdoctoral specialization in microbiology and immunology—also at PSU College of Medicine—was funded through a competitive fellowship award from the National Institutes of Health and focused on studying the role of viruses in cancer. During her nearly two decades as a scientist–educator, she has trained thousands of undergraduate and graduate students pursuing healthcare careers and secured extensive federal funding to promote STEM education and empower underrepresented groups in STEM.

Her considerable STEM program development experience ranges from developing and launching a biomedical sciences baccalaureate program to serving as a curriculum designer and subject matter expert for the Florida Space Research Institute and Workforce Florida. A highlight of her international experience includes work as a speaker for the U.S. Department of State's International Information Programs (IIP). As a part of the IIP program, Dr. Norman-McKay traveled to Tajikistan and Uzbekistan to meet with students, faculty, government officials, community leaders, U.S. diplomats, and other stakeholders to bolster STEM education, empower women and youth in STEM, and help build STEM capacity in central Asia.

Dr. Norman-McKay currently serves as a section editor for the American Society for Microbiology's (ASM) *Journal of Microbiology and Biology Education* and is an active participant in ASM's Microbiology in Nursing and Allied Health Committee. In addition to authoring the first and now second editions of this text, she is a lead author of the *Microbiology Laboratory Theory and Applications* series (Morton Publishing).

Dedication

Although I often get emails from former students, in March of 2020, I received more than usual—and they all had a different tone. These emails were from professionals in medicine who recalled their microbiology training and our discussions of emerging pathogens, especially viruses. They recollected discussions about transmission precautions, biosafety level ratings, vaccines and how they work, and antiviral drugs (to include the limited nature of our arsenal of these drugs). They also knew that the last major pandemic, the 1918 flu pandemic, had killed more Americans in 9 months than World Wars I and II, the Vietnam War, and the Korean War combined. They were scared. They had been mobilized in full force, briefed on new triage protocols, given tips for conserving their personal protective equipment, and advised to keep their distance from family and friends who were at risk for severe disease from "the virus." I replied to each email with a reassurance that while they would likely be pushed to the breaking point, they had the training and constitution to rise to the challenge, and in the balance of it all, they would save lives and ease suffering.

In the first year of the pandemic, COVID-19 killed over 3,600 U.S. healthcare workers. I'm not sure how many former students of mine have become ill or how many have died in this pandemic, but after nearly two decades of teaching *thousands* of prospective nurses and other allied health workers, I must assume a number greater than zero.

This book is dedicated to my students, to the healthcare teams of today and tomorrow, and to the over 115,000 healthcare workers around the world who lost their lives to COVID-19.

With gratitude,
Lourdes Norman-McKay
and all of Pearson Education

Preface

If you're reading this, there's a good chance that you either train tomorrow's healthcare team or you are training to become one of tomorrow's healthcare heroes. In both cases, this pandemic has offered a new lens through which you should view your work. This course is about employable education, learning to think critically and clinically, and seeing how topics integrate so that you are ready to meet and overcome the challenges you'll inevitably face as you advance in your training and eventually your career. This course is about more than a grade—this is a core component of your career readiness. The faculty teaching you are highly trained professionals who will push you to realize your potential. They are your mentors, your cheer squad, and your drill sergeants all in one.

It is through this lens that I and our editorial team have continued the work of setting *Microbiology: Basic and Clinical Principles* apart from other texts as the premier teaching and learning resource built from the ground up, specifically for training tomorrow's healthcare team.

Because this revision was written in the throes of the COVID-19 pandemic, every day has been a vivid reminder to stay true to the goal of supporting an employable educational experience that supports integrated learning as well as clinical and critical thinking. This lens focused *every* aspect of this new edition, literally from cover to cover, on that mission.

When you look at the chapter openers and the cover of the text, you'll see real-life healthcare professionals who, like you, overcame barriers to get through their training. Despite being overworked throughout the pandemic, they took the time to send us pictures of themselves in action so that students could see diverse races and ethnicities, all body types, genders, and ages and know that there is a place for *everyone* in STEM and medicine, regardless of where you come from or what you look like. As you undertake your microbiology journey, you are preparing to join thousands of professionals who have embraced the challenges of always learning, pushed themselves to be kind even when they were exhausted, and in many cases even made the ultimate sacrifice.

To support your important journey, we have designed new e-learning tools called *Interactive Content Reviews* that provide active learning experiences that help you explore and learn and test your knowledge attainment on the most challenging topics in this course. We also added over half a dozen more *Concept Coaches*—an expansion of an already well-loved feature that brings the art to life and helps you see how concepts interconnect. Lastly, the e-revision adds even more quiz and test questions to help you practice the content and master thinking critically and clinically.

We also did a "deep dive" into the text and made important updates across a wide variety of topics to, of course, include extensive coverage of SARS-CoV-2/COVID. Check out the "New to This Edition" section to learn more.

New to This Edition

Chapter 1

- *New Asset:* Concept Coach—The Gram Stain
- In section 1.1, expanded discussion of laws versus theories; revised Figures 1.3 and 1.4 for clarity.
- Revised section 1.2 on classifying microbes for clarity; moved content on biofilms to follow content on the normal microbiota, which was expanded.
- In section 1.3, clarified terminology for primary dye and counterstain; simplified discussion of phase contrast in Table 1.3; clarified discussion of lipopolysaccharide; added a Clinical Vocabulary Note on septic shock.

Chapter 2

- In section 2.2, expanded the discussion of valence electrons, polar covalent bonds, and dipoles; clarified the discussion of Van der Waals interactions and hydrophobic, hydrophilic, and amphipathic molecules; added a Chem Note with line art to clarify the chemical shorthand used in molecular line drawings.
- In section 2.4, added a discussion of polysaccharides to the content on carbohydrate structure.

Chapter 3

- *New Asset:* Interactive Content Review (combined with Chapter 4 to build connections between prokaryotic versus eukaryotic cells)
- New Concept Coach: The Gram Stain
- In section 3.1, increased detail in Figure 3.2 showing prokaryotic cell.
- In section 3.2, added Chem Notes on ether and ester bonds, isoprenoids, and glycans; added a brief explanation of L-form bacteria and the role of Emmy Klieneberger-Nobel; added Figure 3.10 on the structure of peptidoglycan and Figure 3.12 on medically important Gram-negative and -positive bacteria; added details to Figures 3.11 and 3.15.
- In section 3.3, rearranged Figure 3.27 to match presentation in text.

Chapter 4

- *New Asset:* Interactive Content Review (combined with Chapter 3 to build connections between prokaryotic versus eukaryotic cells)
- In section 4.1, provided the word origin for *eukaryotic* to emphasize that these cells are nucleated; revised Figure 4.2 showing eukaryotic cell, adding a prokaryotic cell for comparison.
- In section 4.4, revised Figure 4.20 of the endoplasmic reticulum for clarity; added a new Chem Note on toxic oxygen intermediates.

Chapter 5

- *New Asset:* Interactive Content Review
- In section 5.1, revised Figure 5.1 for clarity and match changes in Chapter 3.
- In section 5.2, explained the naming convention for DNA polymerases; revised DNA replication Figures 5.7 and 5.8 for clarity.
- In section 5.3, revised structure to improve flow of narrative; revised Figure 5.9 to improve readability; changed orientation of tRNA in Figure 5.10 to match revised Figure 5.12 on translation; revised Figures 5.12 and 5.13 for clarity.
- In section 5.5, added explanation of why induced mutations are often harmful to cells.

Chapter 6

- *New Asset:* Interactive Content Review
- Throughout the chapter, added content on SARS-CoV-2.
- In section 6.1, replaced the Training Tomorrow's Health Team with one on coronavirus vaccines; replaced the TEM of herpes virus in Figure 6.2 with one of a coronavirus.
- In section 6.2, added SARS-CoV-2 to Figure 6.8; altered Figure 6.9 to improve size comparison.
- In section 6.3, modestly expanded the discussion of T-even/T4 bacteriophages; revised Figures 6.13 through 6.15 for clarity; rearranged Figure 6.16 to match the order of presentation in the narrative.
- In section 6.4, added content on chloroquine and hydroxychloroquine and the limitation of *in vitro* findings in predicting drug efficacy *in vivo*; replaced the Training Tomorrow's Health Team on egg-free influenza vaccines with one on detecting SARS-CoV-2.

- Moved discussion of prions to new section 6.5; expanded to discuss neurodegenerative diseases associated with misfolded proteins.

Chapter 7

- New Concept Coach: The Bacterial Growth Curve and an Overview of Aerotolerance
- Changed chapter title to Fundamentals of Microbial Growth and Decontamination.
- In section 7.1, reorganized the learning outcomes to better reflect the order of topics.
- In section 7.2, expanded the discussion of antioxidants, and added the term to the glossary; altered Table 7.1 for clarity.
- In section 7.3, added a discussion of hemolysins, and added the term to the glossary; deleted the Training Tomorrow's Health Team on the use of genetics in disease outbreaks.
- Changed the title of section 7.4 to Basics of Microbial Growth Reduction and Decontamination.
- Replaced the Training Tomorrow's Health Team on triclosan with one on decontaminating N95 masks during the COVID-19 pandemic.

Chapter 8

- *New Asset:* Interactive Content Review
- In section 8.1, redrew Figure 8.1 of anabolism and catabolism for clarity; made the chemical drawing of ATP a numbered figure; revised Figure 8.3 (former 8.2) for clarity.
- In section 8.2, expanded the discussion of allosteric regulation; revised the layout of Figure 8.13 to improve comparison of concepts.
- In section 8.4, revised Figures 8.17 through 8.22 to improve visual and textual accessibility, readability, and clarity; revised the discussion of the electron transport chain for clarity.
- In section 8.5, in Figure 8.24 of fermentation, improved visual accessibility and representation of carbon; redrew Figure 8.25 to better align with cellular respiration tabbed headings and to serve as a more detailed summary.
- In section 8.6, revised the discussion of lipid biosynthesis for clarity; revised Figures 8.27 through 8.30 on building organic molecules to better align with cellular respiration tabbed headings and improve visual accessibility and clarity.
- Condensed section 8.8 and simplified Figure 8.31.
- Replaced end-of-chapter review question 15 with a matching-style question.

Chapter 9

- Extensive SARS-CoV-2 content infused throughout the chapter.
- New Concept Coach: Modes of Transmission and the Stages of an Active Infection
- In section 9.1, revised the discussion of Koch's postulates for clarity; deleted Table 9.1 because it repeats material in Chapter 1.
- In section 9.2, expanded the discussion of airborne transmission to compare droplet and aerosol transmission.
- Reorganized section 9.3 for clarity.
- In section 9.4, added discussion of double-blinded placebo-controlled studies; updated Figure 9.6; replaced Figure 9.7 with a graph of COVID-19 hospitalization; added a Training Tomorrow's Health Team on graphing inequality in access to healthcare.
- In section 9.5, updated Figure 9.10 of most common healthcare-acquired infections.
- In section 9.6, updated Figure 9.12, replacing avian flu with COVID-19; expanded the discussion of ethical considerations in vaccination.

Chapter 10

- Throughout the chapter, added content on SARS-CoV-2.
- Reorganized section 10.1 for clarity; added photo of 1918 flu pandemic.

- In section 10.2, revised headings for clarity; expanded discussions of virulence and basic reproduction number (R-naught or R_0) and effective reproduction number (R_e); added a micrograph.
- In section 10.3, revised the discussion of quorum sensing and Figures 10.5, 10.8, and 10.10 for clarity.
- In section 10.4, expanded the discussion of airborne precautions; added a figure on PPE for COVID-19; revised Figure 10.14 (formerly 10.13) on NIOSH-approved respirators.

Chapter 11

- *New Asset:* Interactive Content Review (combined with Chapter 12 to integrate the branches of immunity)
- Revised section 11.1 to introduce immune self-tolerance earlier; revised Figure 11.1 to emphasize innate and adaptive immunity; updated other orienting diagrams to match.
- In section 11.2, mentioned the evolutionary conservation of antimicrobial peptides.
- In section 11.5, added a discussion of cytokine storms; revised Figure 11.11 to improve visual accessibility and clarity.
- In section 11.6, corrected Figures 11.13 through 11.15 and improved visual accessibility and readability.

Chapter 12

- *New Asset:* Interactive Content Review (combined with Chapter 11 to integrate the branches of immunity)
- In section 12.1, added a definition of the term *thymocytes* and expanded the discussion of T cell receptors; revised Figure 12.1 to match the related figure in Chapter 11; updated orienting diagrams to match; revised Figure 12.5 of T cell receptor for clarity.
- In section 12.2, revised discussion of superantigens and T cell activation for clarity.
- In section 12.3, revised the discussion of antibody structure and isotopes to cover constant versus variable regions; revised Figure 12.19 for clarity; added visuals to T-dependent and T-independent descriptions; added visual of full antigen with different epitopes.
- In section 12.4, added a discussion of convalescent plasma and monoclonal antibody therapies; expanded the Training Tomorrow's Health Team on antibody titers to include their role in COVID-19 and plasma donations for emerging infectious diseases; expanded the Bench to Bedside on antibody therapies to explain naming.

Chapter 13

- *New Asset:* Interactive Content Review
- In section 13.1, emphasized the role of vaccine hesitancy in measles resurgence; revised the content introducing autoimmune disorders and the possible role of infection in etiology.
- Reorganized the introduction to section 13.2 for clarity.
- In section 13.3, updated information on allergy management and desensitization.

Chapter 14

- New Concept Coach: An Introduction to Vaccines
- New Concept Coach: Key Molecular Diagnostics 101
- To better represent chapter content, changed chapter title to Biomedical Applications: Vaccines, Diagnostics, Therapeutics, and Molecular Methods.
- In section 14.2, restructured the discussion of vaccine formulations and altered Figure 14.3 on herd immunity for clarity; replaced former Table 14.3 on vaccine formulations with Figure 14.4.
- In section 14.3, deleted discussion of immunoprecipitation reactions and Figures 14.7 and 14.8; combined former Figures 14.10 and 14.11 to improve comparison of indirect, direct, and sandwich ELISA; added Figure 14.12 showing Western blot test result.
- In section 14.4, revised discussion of CRISPR-Cas9 and Figure 14.15 for clarity.

Chapter 15

- New Concept Coach: Antimicrobial Drug Basics
- New Concept Coach: Understanding Antimicrobial Drug Resistance and Drug Stewardship
- In section 15.1, revised and renamed the discussion on modifying antimicrobials.
- In section 15.2, expanded the discussion of the order Enterobacterales.
- In section 15.3, added a discussion of the use of remdesivir for COVID-19 and replaced the Bench to Bedside on antivirals for Zika with one on remdesivir.
- In section 15.5, updated the discussion of resistant microbes.

Chapter 16

- In section 16.1, revised the section on the respiratory microbiome, including Figure 16.4.
- In section 16.2, thoroughly revised the discussions of respiratory syncytial virus and influenza; distinguished between New World and Old World hantaviruses.
- Added COVID-19 content throughout the chapter; the main coverage occurs in section 16.2 and includes new learning outcomes, key terms, table, Disease Snapshot, and Build Your Foundation questions. The SARS map (former Figure 16.6) and Disease Snapshot have been removed.
- In section 16.3, expanded the discussion of diphtheria; updated Figure 16.2 on pertussis; replaced the Bench to Bedside on flu vaccines with one on ventilator-associated pneumonia in COVID-19 patients.

Chapter 17

- Added 40+ photos that show how skin conditions present in people of color; an often-overlooked aspect of dermatology (e-book photo carousel).
- In section 17.2, updated shingles vaccine information to reflect the discontinuation of the Zostavax vaccine and the shift to Shingrix.

Chapter 21

- In section 21.1, expanded the discussion of sepsis and added information on the latest Sequential Organ Failure Assessment (SOFA) for scoring sepsis in patients.
- In section 21.2, replaced map of Ebola outbreak with new Figure 21.10 on Ebola virus transmission; added information about the new Ebola vaccine, Erbevo.
- In section 21.3, updated plague incidence map.
- Restructured section 21.5 on malaria; added that the GlaxcoSmithKline RTS,S/AS01vaccine is now the first ever WHO recommended malaria vaccine to protect children.

Global changes:

- Updated references as needed to communicate the most current developments in microbiology (e.g., new COVID vaccines and Zostavax discontinuation)
- All statistics updated to reflect the most recent data available at time of publication.
- Latest naming conventions applied throughout:
  - *Clostridium difficile* updated to *Clostridioides difficile*
  - Carbapenem-resistant Enterobacterales rather than carbapenem-resistant Enterobacteriaceae. The taxonomic order name, Enterobacterales, is now recommended in place of the family name, Enterobacteriaceae, to reflect that carbapenem resistance is no longer isolated to one family but now spans multiple families within the order.
- Revised and updated section learning outcomes to align with new and/or updated content
- Infused extensive SARS-CoV-2 information throughout the text and media assets.

Continuing to Support DE&I

- Graphing Inequality in Access to Healthcare: TTHT, Chapter 9, page 290
- Novel Vaccines for a Novel Coronavirus (Training Tomorrow's Health Team feature) and Dr. Kizzmekia Corbett: page176
- Encouraging students to build their STEM identity (chapter openers and cover)
- Chapter 17 dermatological images expansion
- Expanded language notes for non-native speakers of English

Acknowledgments

Although my name is on the cover, writing a textbook is not a one-person effort. It takes a team of editors, media specialists, talented artists, and expert reviewers to make a high-quality educational resource that is *thoroughly* peer-reviewed, student-centered, and employability driven. Our team, which was curated from across the globe, is full of talented people.

I especially want to thank Jennifer McGill-Walker for her leadership and willingness to take the "road less traveled" to do things like host a live photoshoot that brought the visages of *real-life* healthcare heroes to every chapter of this text, as well as to the cover. Lara Braun was an instrumental part of the development process—she brought her tireless work ethic, acumen for development, and ever-patient personality to the table. Laura Bonazolli served as our incredibly talented development editor; she is probably the only person on the planet who has read this book more times than me! Matt Walker provided excellent content strategy leadership that brought 2e to the next level. Titas Basu, our content producer, went beyond the call of duty to make the cover-to-cover healthcare heroes vision a reality—overseeing an endless stream of permissions documents and releases, all while coordinating the underlying machinations of seeing a major text revision to market. Hilair Chism, returned to this edition to continue building the unique art style that sheds clutter for clarity and treats art as an avenue to learning.

Mireille Pfeffer, served as our rich-media guru who navigated the perils of the ever-shifting technology world to bring our e-assets to fruition. Kassi Foley worked for over a year with me, Jen, and Lara to shape and pilot the first ever *Interactive Content Reviews* that are an exemplar of how technology can support best practices in teaching. Behind the scenes, Serina Beauparlant is always cheering us on as we shape teaching and learning tools for the world. And then there is Jeanne Zalesky who has been unflagging in supporting our team's work to innovatively conquer the biggest teaching and learning challenges in science higher education. A special thanks to Yez Alayan, Brett Freitas, Kelly Galli-Reynolds, Kate Gittins, Adam Goldstein, Tim Galligan, Jennifer Key, and Tim Wilson––your outreach and support are indispensable. Brandi Bleak, I am grateful for your talented photographer's eye on our cover and many of the chapter openers. Mary Tindle, thanks for being an amazing partner in the production process.

Lastly, I couldn't have finished this work without my husband and best friend, Andrew McKay—nor without the understanding and patience of our amazing daughters, Lourdes Catherine (18) and Delia (13). I love you all more than you can know! Thanks also to my parents for pitching in with a healthy meal when I was up against tight deadlines. And much love to my friend and fellow authoress, Dr. Erin Amerman.

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Contents

1

Introduction to Microbiology

What Will We Explore? You are venturing into a new world, one consisting of extremely small organisms so abundant that they are estimated to comprise at least half of the living biomass on Earth.[1] For thousands of years people were unaware of this invisible microbial landscape. Our veil of ignorance started to lift in the late 1600s, when microscopes were invented, and we saw for the first time the diversity of lifeforms coexisting around us, in us, and on us. In 1665 Robert Hooke first formally described microbial life in his book *Micrographia*, stating, "By the means of telescopes, there is nothing so far distant but may be represented to our view; and by the help of microscopes, there is nothing so small as to escape our inquiry[.]" Since Hooke's time, we have continued expanding our knowledge. This chapter introduces a brief history of the field, then explains how we classify microbes and their interactions. Finally, we discuss the modern microscopes and staining techniques essential to the practice of microbiology today.

Why Is It Important? Understanding microbes is central to understanding human health and disease. These days, even toddlers are taught the connection between microbes and illness. But in recent decades, it has become clearer that our relationship with microbes is more complex than just "germs make us sick." In a way, each one of us is an ecosystem of many hundreds of species of microbes, some of which perform important functions in our bodies like making essential vitamins, training our immune system, and helping us digest food. Microbes also play important roles in sustaining ecosystems, cleaning our global environment, and producing foods, drugs, and even fuels.

Clinical CASE
NCLEX
HESI
TEAS

The Case of the Mystery Pathogen

Visit the **Mastering Microbiology** Study Area to watch the case and find out how microbiology can explain this medical mystery.

Hong Phung
PTA; Jacksonville, FL

[1] Kluyver, A. J., & van Niel, C. B. (1956). *The microbe's contribution to biology*. Cambridge, MA: Harvard University Press, p. 3.

1.1 A BRIEF HISTORY OF MICROBIOLOGY

Learning Outcomes

After reading this section, you should be able to:

1.1 Define the term *microorganism*, and give examples of microbes studied in microbiology.

1.2 Explain the distinction between a pathogen and an opportunistic pathogen.

1.3 Compare the theories of biogenesis and spontaneous generation, and summarize Louis Pasteur's role in proving biogenesis.

1.4 Describe how Robert Koch helped shape the germ theory of disease, and list his postulates of disease.

1.5 Identify the goals of aseptic technique, and explain why it is important in healthcare facilities and laboratories.

1.6 Discuss how Semmelweis, Lister, and Nightingale contributed to health care.

1.7 Outline the basic steps of the scientific method, distinguish an observation from a conclusion, and compare a scientific law to a theory.

S.M.A.R.T. Strategy

Visit the **Mastering Microbiology** Study Area to watch the Concept Coach and learn a useful strategy for thinking clinically and critically.

CONCEPT COACH NCLEX HESI TEAS

What is microbiology?

Microbiology is the study of **microbes**, which are often invisible to the naked eye. The term *microbe* encompasses cellular, living microorganisms such as bacteria, archaea, fungi, protists, and helminths, and nonliving/noncellular entities such as viruses and prions (infectious proteins) (TABLE 1.1). Some microorganisms are not microscopic. For example, a number of fungi, helminths such as parasitic worms, and protists such as algae are visible to the naked eye.

At least half of Earth's life is microbial. Microbes inhabit almost every region of our planet, from deep-sea trenches to glaciers. And we still have much to discover, as it is estimated that less than 1 percent of the world's microbes are currently identified.

Evolving about 3.5 billion years ago, **prokaryotic cells** (PRO-care-ee-ah-tic) are Earth's earliest lifeforms. They include unicellular bacteria and archaea (are-KEY-uh), which are structurally and functionally simpler than **eukaryotic cells** (YOU-care-ee-ah-tic). Eukaryotic cells make up all multicellular organisms and a number of unicellular microorganisms such as amoebae and yeast. The **endosymbiotic theory** states that eukaryotic cells evolved from prokaryotic cells. (For more on prokaryotic cells, eukaryotic cells, and endosymbiotic theory, see Chapters 3 and 4.)

Microbiology spans a wide variety of fields, including health care, agriculture, industry, and environmental sciences. Humans rely on microbes for many things such as food production, making medications, and breaking down certain environmental hazards.

A great deal of microbiology research focuses on **pathogens**—microbes that cause disease. However, most microbes are not pathogens. Just over 1,400 pathogenic microbes are known to infect humans;[2] but overall, less than 1 percent of all microbes are likely to be pathogenic. Among pathogens, we find so-called

TABLE 1.1 Living and Nonliving Agents Studied in Microbiology

Microbe	Cell Type	Notes
Bacteria	Prokaryotic	Unicellular;* pathogenic and nonpathogenic
Archaea	Prokaryotic	Unicellular; nonpathogenic; most live in extreme environments
Protists	Eukaryotic	Unicellular and multicellular; pathogenic and nonpathogenic (unicellular example: amoebae; multicellular example: algae)
Fungi	Eukaryotic	Unicellular and multicellular; pathogenic and nonpathogenic (unicellular example: yeast; multicellular example: mushrooms)
Helminths	Eukaryotic	Multicellular;* parasitic roundworms and flatworms
Viruses	Not cells; nonliving	Infect animal, plant, or bacterial cells; can have a DNA or RNA genome
Prions	Not cells; nonliving; infectious proteins	Not discovered until the 1980s; transmitted by transplant or ingestion; some prion diseases are inherited

*__Unicellular__ = one-celled organism; __multicellular__ = organism made of many cells

[2] Woolhouse, M. E. J., & Gowtage-Sequeria, S. (2005). Host range and emerging and reemerging pathogens. *Emerging Infectious Diseases*, *11*(12), 1842–1847.

true pathogens, which in theory can cause disease in any otherwise healthy host, and we find **opportunistic pathogens**, which tend to cause disease only in a weakened host. Typically, microbiology laboratories have the task of identifying the specific microbe causing a patient infection.

Great advances occurred in and around the golden age of microbiology.

The golden age of microbiology (approximately 1850–1920) was sparked by innovations in microscopes and new techniques to isolate and grow microbes (FIG. 1.1). Many of the techniques that facilitated this turning point in microbiology are still used today.

FIGURE 1.1 Select events in the early history of microbiology

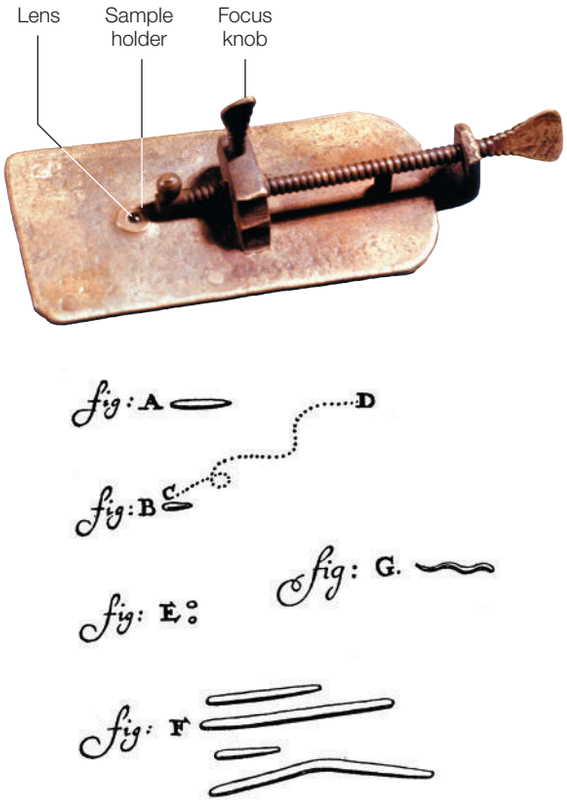


FIGURE 1.2 First views of bacteria Antonie van Leeuwenhoek was the first to report descriptions of bacterial cells. *Top*: Antonie van Leeuwenhoek used a small handheld microscope that had at best a 300 × magnification capability. *Bottom*: Leeuwenhoek's drawings of "very little animalcules."

CHEM • NOTE

Fermentation is a chemical process that often leads to the production of acids and/or alcohols. Fermentation has long been exploited by people who tap microbes to create wine, beer, and vinegar, among other food products.

Spontaneous Generation versus Biogenesis

In the mid-1600s, Robert Hooke used crude microscopes to view a variety of tiny structures, from fleas to snowflakes, and became the first scientist to publish descriptions of cells. Antonie van Leeuwenhoek, a contemporary of Hooke, refined earlier versions of the microscope and became the first to see bacteria (FIG. 1.2). Like many of their contemporaries, these scientists participated in the debate about **spontaneous generation**, an idea that life comes from nonliving items, and **biogenesis**, the idea that life emerges from existing life.

Several other 17th-century scientists, including Francesco Redi, performed experiments to test the hypothesis of spontaneous generation. One piece of evidence often cited as proof of spontaneous generation was that rotting meat gave rise to maggots. To further explore this evidence, Redi placed one piece of meat in an uncovered jar and a second piece of meat in a jar with a gauze-covered top. The uncovered meat gave rise to maggots because flies could lay their eggs on it. The meat in the covered jar did not give rise to maggots, because flies were unable to touch the meat.

One would think Redi's experiments would have debunked spontaneous generation, but the theory persisted for another 200 years until the late 1800s, when Louis Pasteur showed that biogenesis is responsible for the propagation of life. Before his experiments, stored wine often fermented and turned bitter. A common explanation for the bitterness was spontaneous generation of yeast during the fermentation process. Pasteur proved that yeast *performed* fermentation—they were not spontaneously generated by it. He showed that by heating new wine to 50–60°C, he could kill off the yeast within it and delay spoilage for years. We now know this heating process as **pasteurization**. It is most commonly used to treat milk and juices to render these foods safe for consumption and to slow spoilage.

Convinced that air contained contaminating microbes, Pasteur further investigated his hypothesis by performing an experiment with a specialized S-necked flask that was partially filled with broth (FIG. 1.3). He boiled the broth and showed

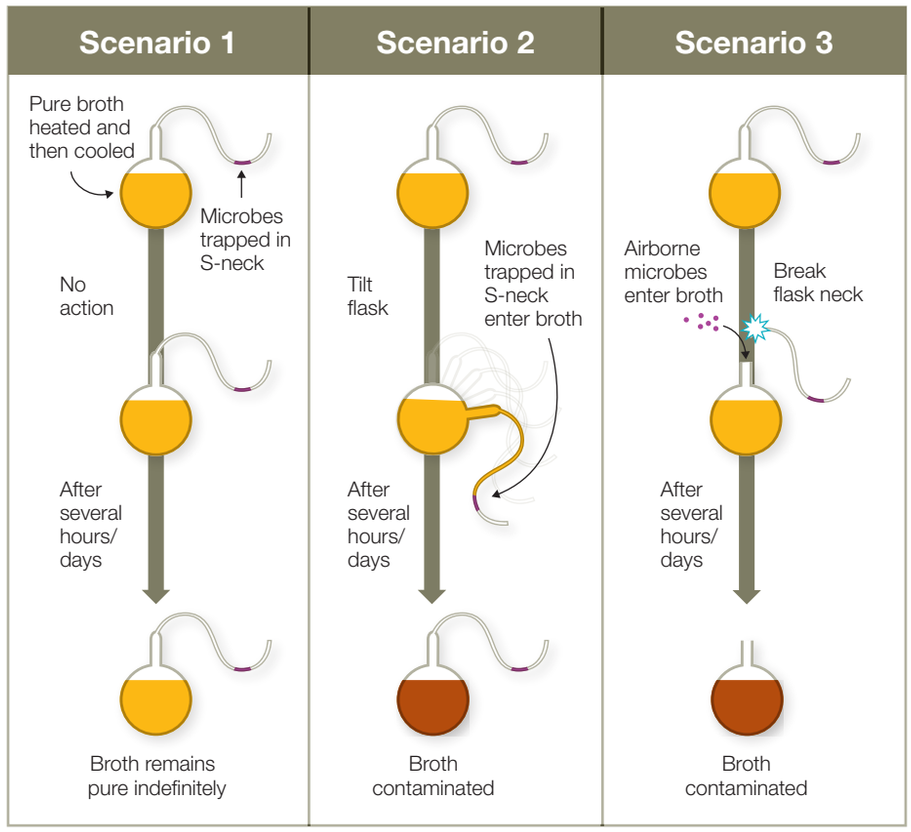


FIGURE 1.3 Pasteur's experiment Louis Pasteur's S-necked flask experiment disproved spontaneous generation.

Critical Thinking *Why was it important that the broth was heated in the same flask as it was cooled?*

that it remained unspoiled because microbes in the air were trapped in the bent portion of the flask, unable to reach the liquid below. When the flask was shaken, broth encountered the microbes previously trapped in the curved neck, and the broth would then spoil. Pasteur's work went beyond disproving spontaneous generation. He also developed the first vaccines to protect against anthrax and rabies (two deadly diseases that can be transmitted to humans from other animals) and he had a significant role in solidifying the germ theory of disease.

Germ Theory of Disease

The **germ theory of disease** states that microbes cause infectious diseases. From the late 1800s forward, determining the specific **etiological**, or causative, agent of an infectious disease became an important role of microbiology labs. Despite over a century of research, we are still nowhere near discovering the specific etiological agent of every infectious disease. We'll probably never have a complete catalog of every infectious agent, because even as we make progress identifying them, new diseases emerge. Further complicating matters is the fact that current laboratory techniques enable us to grow only about 2 percent of the bacterial species found in our environment.

T. pallidum

Although laboratory limitations and the evolving nature of microbes make characterizing the etiological agents of disease more challenging, it does not make it impossible. For example, the bacterium *Treponema pallidum* was discovered to be the cause of syphilis over 100 years ago, yet it was only in 2018 that the bacterium was successfully cultured *in vitro* (meaning "in glass," or in an artificial setting).[3] Prior to 2018, it had only been sustained *in vivo* (meaning "in the living"—in animal models) using rabbits.

Louis Pasteur and his contemporary Robert Koch (pronounced "coke") both reinforced the germ theory of disease. Koch was a German physician who developed staining techniques and ways to grow and isolate bacteria. Some of Koch's most groundbreaking work started with the study of anthrax, which primarily infects grazing animals but, as mentioned earlier, can infect humans. (For more on anthrax, see Chapter 17.) Koch discovered that anthrax is caused by a bacterium, which he named *Bacillus anthracis*. He was able to isolate *Bacillus anthracis* from diseased animals, then introduced the purified bacteria into mice to establish an infection.

Koch's Postulates of Disease

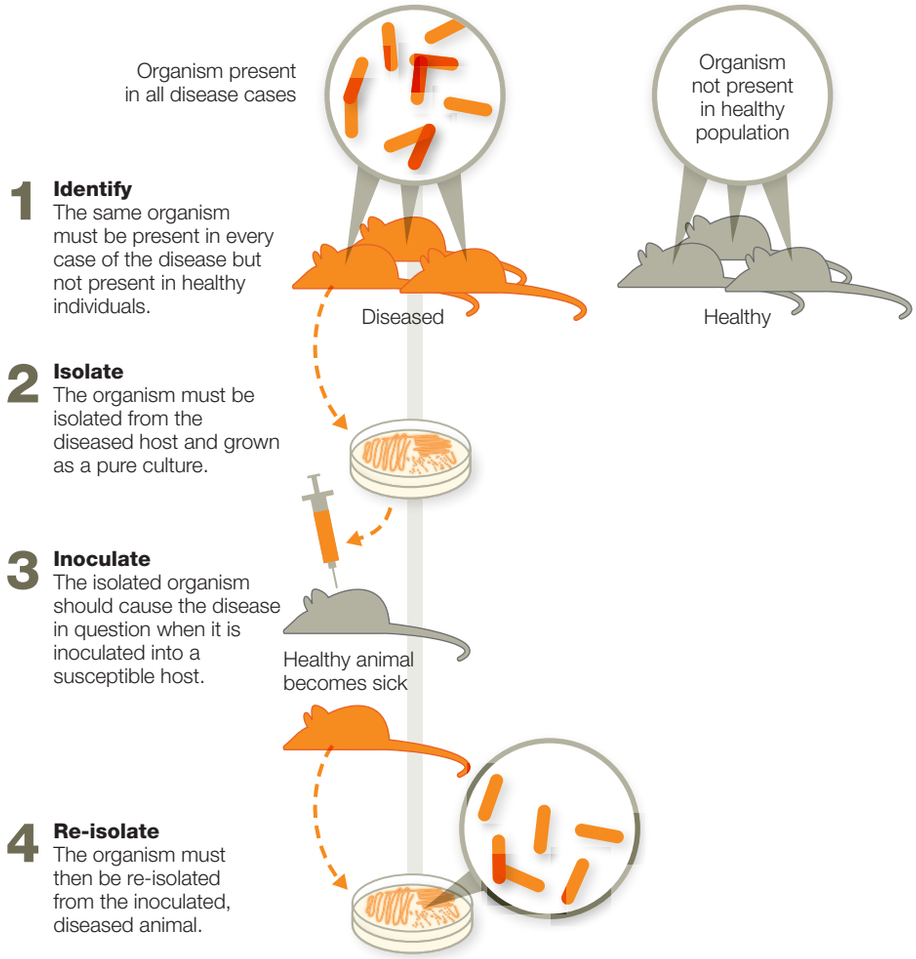
Koch's work on anthrax led to the development of **Koch's postulates of disease** (**FIG. 1.4**). These four principles establish the criteria for determining the causative agent of an infectious disease. (Koch's postulates are briefly listed here, but are discussed in more detail in Chapter 9.)

1. The same organism must be present in every case of the disease.
2. The organism must be isolated from the diseased host and grown as a pure culture.
3. The isolated organism should cause the disease in question when it is introduced (inoculated) into a **susceptible host** (a host that can develop the disease).
4. The organism must then be re-isolated from the inoculated, diseased animal.

[3] Edmondson, D. G., Hu, B., & Norris, S. J. (2018). Long-term in vitro culture of the syphilis spirochete *Treponema pallidum* subsp. pallidum. *MBio*, 9(3), e01153-18.

FIGURE 1.4 Koch's postulates of disease Koch's postulates can help identify the causative agent of certain infectious diseases.

Critical Thinking *It is not ethical to purposefully expose people to a suspected pathogen to fulfill Koch's third postulate. With this in mind, how could the third step be ethically observed if the disease being studied only occurred in humans and an animal model could not be used?*



In his studies, Koch confirmed that not all infections cause evident disease. This is why the third postulate states that disease *should* result, but avoids the term *must* as is found in his other statements.

Hand Hygiene and Aseptic Techniques

From the 1800s through early 1900s, as the biogenesis theory and the germ theory of disease were being developed and debated, several medical professionals were emphasizing the importance of aseptic techniques. In a medical setting, **aseptic technique** entails preventing the introduction of potentially dangerous microbes to a patient; it doesn't mean that everything in the environment needs to be **sterile** (absent of all microbes). While most surfaces in an operating room or other healthcare setting are disinfected to limit potentially dangerous microbes, they are not sterile. Aseptic procedures are central to health care because they prevent **healthcare-acquired infections** or **HAIs** (also known as **nosocomial infections** or **healthcare-associated infections**) and limit the spread of diseases.

Aseptic procedures also allow us to safely study microbes in the laboratory; moreover, they are necessary for maintaining pure samples so that we can study one microbe at a time. The type of aseptic technique used depends on the situation, but aseptic procedures usually include washing hands, wearing gloves, sterilizing instruments, and decontaminating surfaces. (See Chapter 10 for more on healthcare biosafety precautions.)

A Hungarian physician named Ignaz Semmelweis first developed aseptic techniques in the 1840s. He recommended that physicians and other care providers practice hand washing to decrease mortality rates from childbed fever

(puerperal sepsis), an infection that killed many women in childbirth before the antibiotics era.

Semmelweis saved many lives, yet his work was not fully appreciated until about 20 years later, when Pasteur disproved spontaneous generation. Work by Semmelweis and Pasteur inspired the British surgeon Joseph Lister to investigate processes for aseptic surgery. Lister's work in the 1860s proved that sterilizing instruments and sanitizing wounds with carbolic acid encouraged healing and prevented pus formation. Around the same time, Florence Nightingale established the use of aseptic techniques in nursing practices, which, along with other patient-care innovations, led to her being recognized as the founder of modern nursing.

The scientific method is the guiding investigative principle for microbiology.

Before the modern age, illness was often attributed to evil spirits or sinfulness. As such, early medical treatments almost always included some form of penance, pilgrimage, or protective charm. Early physicians thought illness derived from an imbalance of the body's humors that could be relieved by bloodletting or applying leeches to the body (FIG. 1.5).

Today, by contrast, we explore questions about the origins of diseases and potential treatments through the **scientific method**. In its most basic form, the scientific method starts with a question that can be investigated. Next, a **hypothesis**—a prediction based on prior experience or **observation**—is proposed as a potential answer to the question. The researcher collects and analyzes observations (data) and uses them to formulate a **conclusion** that states whether the data supported or contradicted the hypothesis.

Observations versus Conclusions

Failure to recognize the difference between observations and conclusions leads to errors and confusion. An observation is any data collected using our senses or instrumentation, whereas a conclusion interprets observations. For example, suppose you witnessed a robber driving a getaway car with a Florida license plate. If you tell the detective that the robber is from Florida instead of saying the robber's car had a Florida plate, you might mislead the investigation. This scenario is an example of *inference–observation confusion*—more commonly known as "jumping to conclusions." In contrast, science requires a collection of observations and many different methods of testing to draw accurate conclusions. It is essential that healthcare workers avoid inference–observation confusion because it can lead to an inaccurate assessment of the patient. We should also recognize the limits of experimental design and what we can truly conclude from our observations.

Law versus Theory

The difference between a scientific law and a scientific theory also confuses many students. A **law** is a precise statement, or mathematical formula, that predicts a specific occurrence. Laws only hold true under carefully defined and limited circumstances. By contrast, a **theory** is a hypothesis that has been proven through many studies with consistent, supporting conclusions. Laws predict *what* happens, while theories explain *how* and *why* something occurs. Furthermore, theories encompass laws. For example, the theory of evolution, which explains how and why organisms change over time, includes a number of guiding laws such as natural selection promoting the survival of the fittest. Unlike a hypothesis, which focuses on a specific problem, theories are comprehensive bodies of work that are useful for making generalized predictions about natural phenomena. Theories unite many different hypotheses and laws.

CHEM • NOTE

Carbolic acid (C_6H_6O), also known as phenol, is an organic molecule with antiseptic (degerming) and anesthetic (numbing) properties. It can be found in sore throat sprays, lip balms, and various household cleaners, and is also used for making plastics. Phenol can be commercially produced but also occurs naturally in many foods. Concentrated phenol is toxic to humans and is regulated.

FIGURE 1.5 Bloodletting For about 3,000 years, bloodletting was practiced as a primary medical therapy for practically every ailment.

Sometimes people think that laws must be superior to theories—likely because of the way laws are defined and enforced within governments. But one should not equate the social definition of a law with the scientific one. In science, laws and theories have completely different goals and facilitate discovery in different ways. Thus, the idea that a theory would be "elevated" to a law is inaccurate; to do so would be like turning an apple into an orange. Moreover, neither laws nor theories are considered final. Scientists continue to retest laws and theories as our technology and knowledge increase.

Build Your Foundation

1. Microbiology is the study of living and nonliving microscopic entities. Explain. (LO 1.1)
2. What is a pathogen, and how is it different from an opportunistic pathogen? (LO 1.2)
3. Distinguish between biogenesis and spontaneous generation, and discuss how Pasteur disproved spontaneous generation. (LO 1.3)
4. List Koch's postulates of disease, and describe how they contributed to the germ theory of disease. (LO 1.4)
5. The term *aseptic* does not mean 100 percent sterile. Explain why. (LO 1.5)
6. How did Lister, Semmelweis, and Nightingale contribute to health care? (LO 1.6)
7. In a lab report, a student wrote that a red color developed in the test tube being used. Is this an observation or a conclusion? Explain. (LO 1.7)
8. How is a scientific law different from a theory? (LO 1.7)

Build Your Foundation (BYF) Quick Quiz: Visit the **Mastering Microbiology** Study Area to quiz yourself.

1.2 CLASSIFYING MICROBES AND THEIR INTERACTIONS

Learning Outcomes

After reading this section, you should be able to:

1.8 Summarize the taxonomic hierarchy from domain to species.
1.9 Define the term *strain*.
1.10 Describe the binomial nomenclature system and the information it provides about an organism.
1.11 Define the terms *parasitism*, *mutualism*, and *commensalism*.
1.12 Define the term *normal microbiota*, and discuss its establishment and roles.
1.13 Describe a host–microbe interaction that influenced human evolution.
1.14 State how a biofilm forms, and discuss the healthcare implications of biofilms.
1.15 Provide examples of how microbes affect industry and the environment.

Taxonomy groups organisms.

Microbial classification is important both for study and for clear communication among researchers and healthcare providers. **Taxonomy** is the study of how organisms can be grouped by shared features—to include physical features (**morphology**) and physiological characteristics. In the mid-1700s, Carl Linnaeus established criteria for classifying and naming organisms. He is now recognized worldwide as the father of taxonomy.

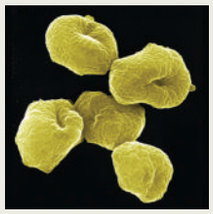
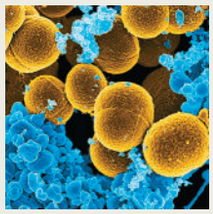
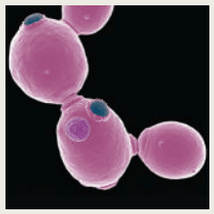
Taxonomic Hierarchy

There are eight rankings within the taxonomic hierarchy. From broadest to narrowest they are: *d*omain, *k*ingdom, *p*hylum, *c*lass, *o*rder, *f*amily, *g*enus, species. The mnemonic device, "*D*elightful *K*ing *P*hilip came *o*ver *f*or *g*reat spaghetti" may help you remember the taxonomic hierarchy.

Domains The broadest category, **domain**, primarily groups organisms by cell type. There are three domains: Bacteria, Archaea, and Eukarya. Domains Bacteria and Archaea encompass prokaryotic organisms—all of which are unicellular and lack a nucleus (an intracellular structure that houses a cell's genetic information). Most of the prokaryotes you will learn about in this text belong to the Domain Bacteria—this grouping includes potential pathogens.

Members of Domain Archaea are best known for living in extreme environments: high-temperature deep-sea vents, areas of bitter cold, or environments with harsh chemical conditions usually devoid of other lifeforms. However, they can also live in normal environments and are also found in the human gut and on our skin. To date, Archaea members have not been shown to be pathogens.

TABLE 1.2 Six-Kingdom Classification System

Kingdom	Archaea	Bacteria	Fungi	Plantae	Animalia	Protists*
Example						
	Sulfolobus	*S. aureus*	*Candida albicans*	(Plants)	(Animals)	*Paramecium*
Domain	Archaea	Bacteria	Eukarya	Eukarya	Eukarya	Eukarya

*Not a true kingdom; a catchall category for lifeforms formerly grouped in Kingdom Protista

The Domain Eukarya encompasses unicellular and multicellular organisms that are made of eukaryotic cells—that is, cells that have a distinct nucleus. There are four main types of eukaryotic cells: animal, plant, fungal, and protist; you'll see versions of these names in the next section on kingdoms.

Kingdoms Beneath the umbrella of domains are a variety of **kingdoms**, the designated number of which has fluctuated from five to eight. The older five-kingdom classification scheme includes the eukaryotic kingdoms Animalia, Plantae, Fungi, Protista, and the prokaryotic kingdom Monera. The trouble with the five-kingdom scheme is that it fails to assign separate kingdoms for Domain Archaea and Domain Bacteria, instead lumping these prokaryotic domains together into Kingdom Monera.

The six-kingdom schematic, which is what this text employs, replaces Kingdom Monera with Kingdom Archaea and Kingdom Bacteria (TABLE 1.2). This scheme also isn't perfect: Traditionally, Protista was a sort of miscellaneous catchall kingdom for organisms that couldn't be described as plants, animals, or fungi. Genetics research now shows that protists can't logically be lumped into a single kingdom. However, the term "Kingdom Protista" persists, and for convenience's sake, this book also continues to informally refer to these diverse organisms as protists. To the ardent taxonomist, this is perhaps a heretical approach, but the goal here is not for you to become a taxonomy expert, just to explore these important lifeforms without feeling overwhelmed by the constantly changing world of taxonomy.

The disagreement on kingdoms emerges because taxonomy has become more than just the grouping of organisms with shared features. Because of new genetic analysis techniques, taxonomy is now often used as a tool to understand how organisms are evolutionarily related. There are a variety of styles for grouping organisms by relatedness in "the tree of life" and there likely will never be a unanimous agreement as to which tree is the best.

Phyla Through Species Regardless of how many kingdoms people acknowledge, each can be further subdivided into smaller and smaller groups: first phylum (phyla = plural); then class, order, family, and genus (genera = plural); and finally into the most precise grouping, species (FIG. 1.6).

Although each of these categories is useful, genus and species are arguably the most common groupings you'll encounter in an introductory microbiology course. You can think of a **genus** as a group of related species. For example, the genus *Canis* includes wolves (*Canis lupus*) and dogs (*Canis familiaris*). Traditionally, a species is defined as a group of similar organisms that sexually reproduce together, or breed. Such a definition doesn't work for prokaryotic cells like bacteria because they reproduce asexually. Instead, **prokaryotic species** are defined as cells that share physical characteristics, but also have at least 70 percent DNA similarity (based on the degree to which their DNA can stably pair

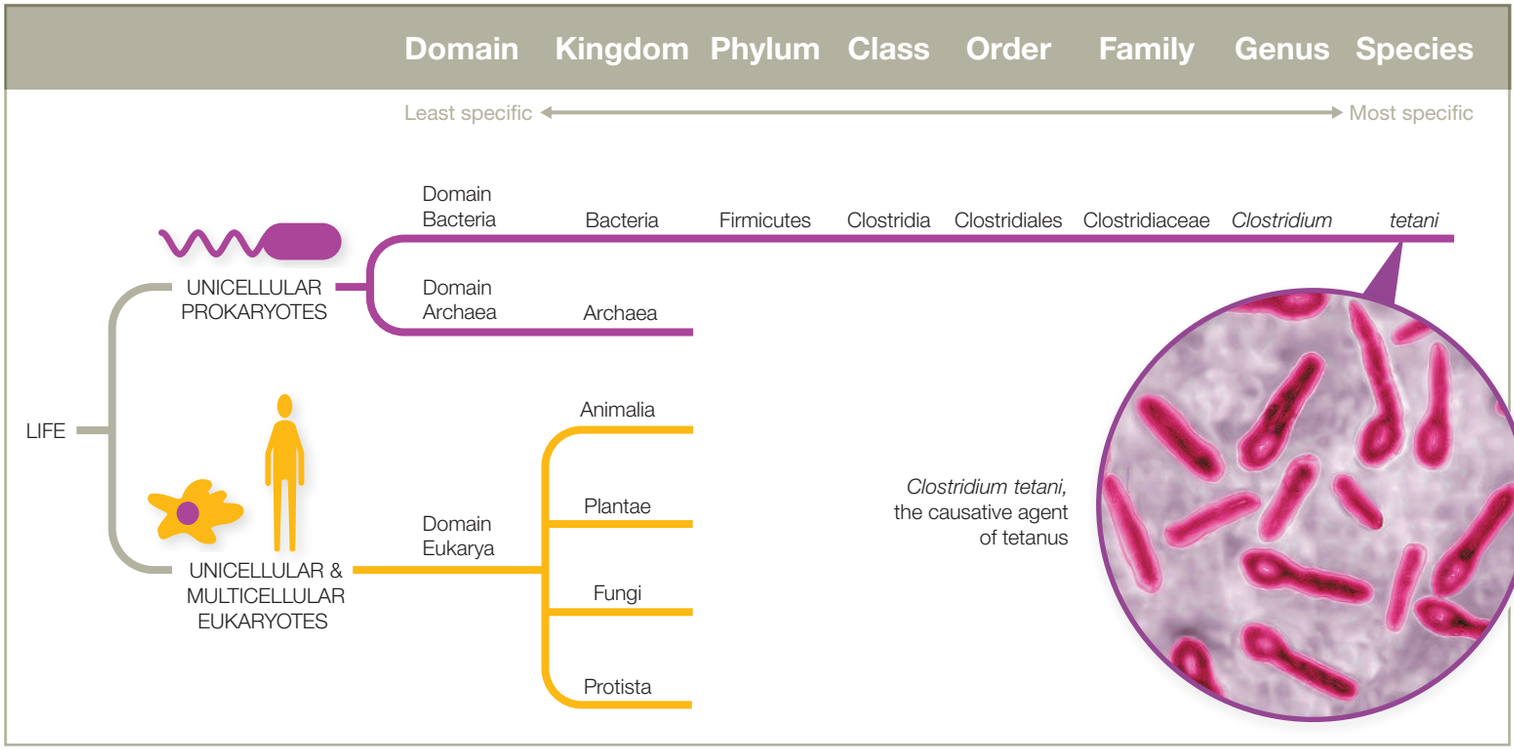


FIGURE 1.6 Classifying organisms This figure shows the taxonomic hierarchy based on a three-domain and six-kingdom system. Domains can be systematically broken down until the most specific ranking, species, is reached. The classification hierarchy for the causative agent of tetanus, *Clostridium tetani*, is shown as an example. *Note that Protista isn't a kingdom in the classical sense, but a catchall category that persists as a matter of convenience.

with each other in solution).[4] Also, prokaryotic species are at least 97 percent identical when genetic material in their ribosomes (organelles that build proteins) is compared.[5]

Strains Many microbiologists argue that trying to systematically identify prokaryotic species might be an exercise in futility because prokaryotes transfer genes and can take up genetic material from their environment. Therefore, recognizing genetic variants is helpful to microbiologists. The term **strain** is used to recognize genetic variants of the same species. Different strains of a species typically have a hallmark genetic trait that warrants a special designation. Mutations and gene transfer often lead to new strains.

As you can see, the parameters for bacterial classification are diverse. Fortunately, the Society of American Bacteriologists (now the American Society for Microbiology, or ASM), has worked to unify the classification criteria for bacteria. Their efforts laid the foundation for *Bergey's Manual of Determinative Bacteriology*. *Bergey's Manual*, as it is often called, has evolved through numerous updates, but remains a cornerstone reference for bacterial identification and classification.

[4] Wayne, L. G., Brenner, D. J., Colwell, R. R., et al. (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *International Journal of Systematic Bacteriology*, 37(4), 463–464; Tindall, B. J., Rosselló-Móra, R., Busse, H. J., Ludwig, W., & Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *International Journal of Systematic and Evolutionary Microbiology*, 60(1), 249–266.

[5] Ribosomes are made of RNA (ribonucleic acid) and proteins. Ribosomal RNA (rRNA) sequences change very little over time, which is why they can be used to determine evolutionary relatedness. Prokaryotic organisms that are closely related will be almost identical in their 16S rRNA sequences—one of several types of rRNA in prokaryotic ribosomes. In Chapter 5, Genetics, you will learn more about rRNA and ribosomes. Stackebrandt, E., & Goebel, B. M. (1994). Taxonomic note: A place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic Bacteriology*, 44(4), 846–849.

Scientific Names

As a part of his work in taxonomy, Linnaeus also established a **binomial nomenclature system**, or two-name system, that is still used today. In this system, an organism's first name is capitalized and reflects the genus, whereas the second name is lowercase and designates the species. Scientific names are also italicized (or underlined if handwritten). When first referring to an organism in writing, the genus and species names are written out in full, and thereafter the name is usually abbreviated by using the capitalized first letter of the genus name, followed by a period, and then the lowercase species name in full. For example, *Staphylococcus aureus* can be written as *S. aureus* once the full name has been noted.

Often scientific names are also Latinized, meaning the names are Latin-sounding; in most cases they are nothing near real Latin. For example, in the name of the bacterium *Escherichia coli*, the genus name derives from the discoverer's name, Theodor Escherich; the species name *coli* reflects that the organism is abundant in the colon. Often a microbe's name refers to its discoverer, cell shape, cell arrangement, or other distinct traits that the person who coined the name found noteworthy. Occasionally, even narrower categories such as strain or subspecies may be noted after the species name. Strain names typically include numbers and/or letters after the species name. For example, a strain of *Escherichia coli* commonly found in laboratories is *E. coli* K-12.

Microbes may be friends or foes.

Some people think of all microbes as a pestilence to be eliminated. However, microbes constitute such a huge part of the Earth's biomass that eliminating them would be an ecological disaster. It is suspected that there are several million species of microbes in our world, but so far just over 7,000 microbes have been characterized.[6] One study showed that there are about 150 different species of bacteria just on the palms of our hands.[7] Fortunately, most microbes are helpful or neutral to human health. Only a small minority are pathogens.

Host–Microbe Interactions

A **symbiotic relationship** exists when two or more organisms are closely connected. Humans and dogs, for example, have a symbiotic relationship. Microbes and their human hosts have evolved a variety of symbiotic relationships, including those that hurt the host (**parasitism**), help the host (**mutualism**), or have no perceived benefit or cost to the host (**commensalism**).

Pathogens are described as having a parasitic relationship with their host. However, the term **parasite** is most commonly used to refer to specific organisms such as helminths (worms) and protozoans (certain protists), which are eukaryotic infectious agents with complex life cycles.

The relationship between humans and our **normal microbiota** (the collection of all the microbes that reside in and on the human body) was traditionally described as commensal. But as we learn more, it is increasingly obvious that our ecological relationship with our normal microbiota is often mutualistic—we benefit from our microbiota and they benefit too.

Indeed, our close ecological relationships with microbes—from our normal microbiota to other microbes encountered in our environment—have led humans and microbes to coevolve. The same is true of the interaction between microbes and other organisms. A classic example of pathogen influence on human evolution is seen with malaria. This mosquito-borne, tropical disease has plagued humans for at least 100,000 years. According to the World Health Organization (WHO),

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[6] Achtman, M., & Wagner, M. (2008). Microbial diversity and the genetic nature of microbial species. *Nature Reviews Microbiology*, *6*(6), 431–440.

[7] Fierer, N., Hamady, M., Lauber, C. L., & Knight, R. (2008). The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *Proceedings of the National Academy of Sciences of the USA*, *105*(46), 17994–17999.

TRAINING

TOMORROW'S HEALTH TEAM

E. coli Strains: Little Differences Have Big Consequences

SEM of *E. coli* O157:H7, one of the causative agents of HUS.

Escherichia coli, the agent of much-publicized foodborne disease outbreaks, is also a normal resident of the human gut. Given that, why aren't more people suffering from the terrible symptoms generally associated with an *E. coli* infection?

The answer is that there are hundreds of *E. coli* strains, and most are harmless. But some, such as *E. coli* O157:H7, cause severe illness. The first recognized *E. coli* O157:H7 outbreak occurred in 1982 and was linked to undercooked hamburgers. However, more recent outbreaks have been associated with fruits and vegetables rather than meat.

The CDC estimates that up to 8 percent of *E. coli* O157:H7 infections result in hemolytic uremic syndrome (HUS), which leads to kidney failure. *E. coli* O157:H7 also makes a toxin known as Shiga-like toxin thanks to a gene that it acquired from *Shigella* species that cause dysentery. From a healthcare standpoint, rapidly identifying the culprit strain of an infection can be the difference between life and death for the patient. For example, studies have shown that treating *E. coli* O157:H7 with antibiotics may actually increase the risk of developing HUS by up to 17 times. The reason for this effect might be that the antibiotic damages the bacteria, causing a sudden and concentrated burst of toxin to be released. Instead of prescribing antibiotics, patients infected with *E. coli* O157:H7 should receive early administration of intravenous fluids, which may help protect the patient from HUS, or limit the damage of HUS.

QUESTION 1.1

Imagine you are a nurse caring for a child with an E. coli *O157:H7 infection. The patient's mother is concerned that an antibiotic is not being prescribed. She says that last year she received antibiotics for a urinary tract infection due to* E. coli. *How would you explain why the child needs different treatments against* E. coli?

malaria kills over 600,000 people every year. People who carry the gene for sickle cell anemia, a blood disorder characterized by a mutation in the gene for hemoglobin, are less likely to develop serious disease if infected by the protozoan pathogens that cause malaria. For this reason, carriers of the sickle cell gene have a survival advantage in areas where malaria is common. This helps explain why in some malaria-plagued regions of sub-Saharan Africa, up to 40 percent of tribal people carry the gene. People originating from parts of the world where malaria incidence is low rarely carry the sickle cell gene.

Normal Microbiota and the Human Microbiome

As previously mentioned, our normal microbiota—also referred to as our normal flora or the human microbiome—consists of all the microbes that reside in and on the human body. Our normal microbiota include bacteria, archaea, and eukaryotic microbes. They train our immune system, produce vitamins for us, and help us digest foods. Studies suggest that they may even impact our moods and brain function.[8] The *Human Microbiome Project* (HMP), an effort sponsored by the U.S. National Institutes of Health, was initiated to study the human microbiome and shed light on the links between these resident microbes and pathological conditions such as obesity, food allergies, heart disease, multiple sclerosis,

[8] Rea, K., Dinan, T. G., & Cryan, J. F. (2020). Gut microbiota: A perspective for psychiatrists. *Neuropsychobiology*, *79*(1–2), 50–62.

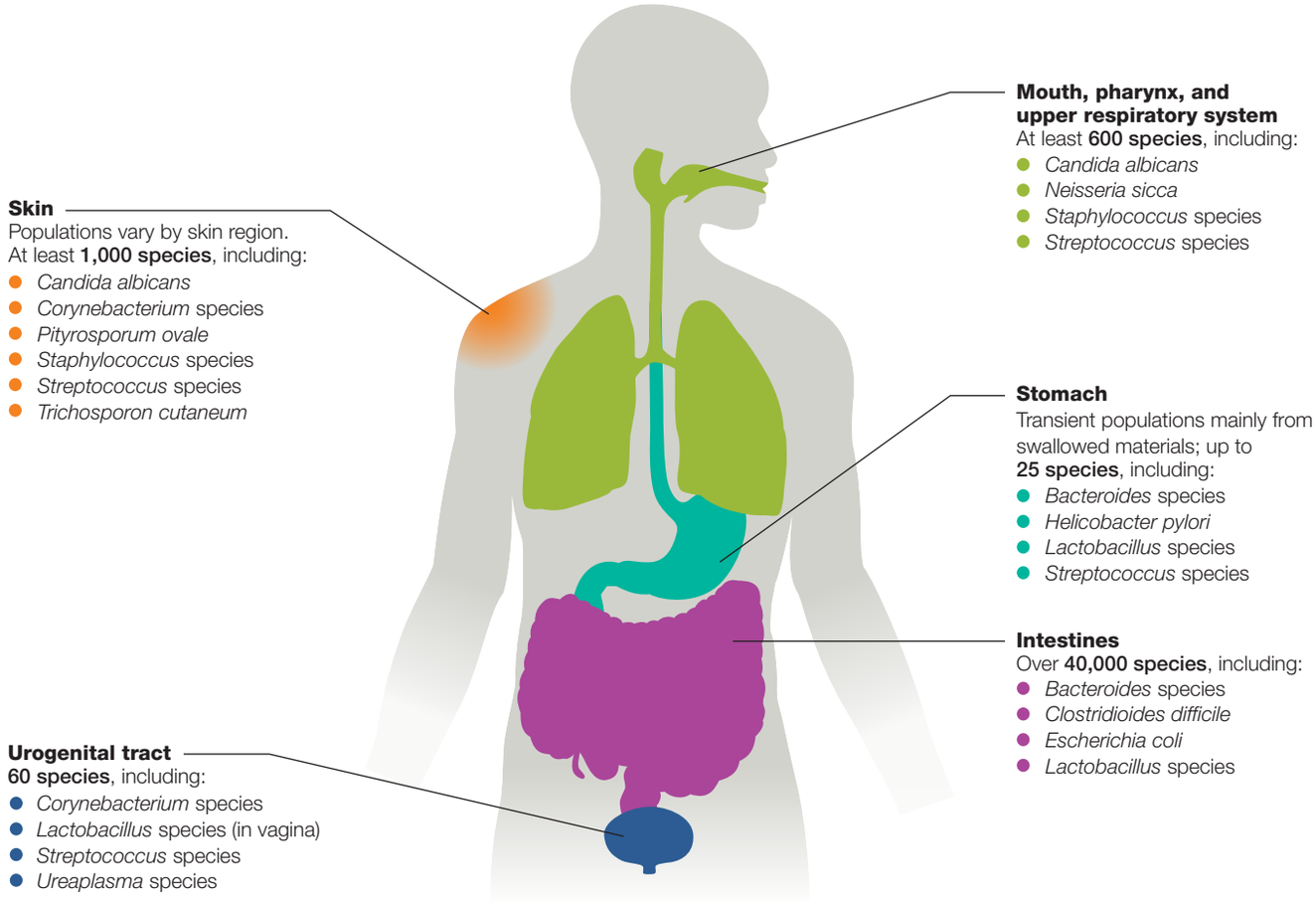


FIGURE 1.7 Our normal microbiota The human microbiome project is characterizing the microbes that call us home. Note the diversity and staggering variety of microbes in and on our bodies.

Critical Thinking *Based on the data presented in the figure, you can see that certain groups of microbes prevail in one body area over another. For example, the streptococci are much more abundant in the mouth than in other body regions. Why do you think microbes dominate a particular region of the body over another?*

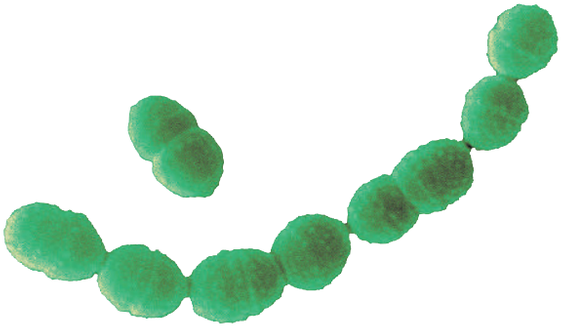
and diabetes. Many parts of the human body teem with microbial life; there are at least as many microbial cells in and on us as there are human cells.[9]

Our skin, nose, mouth, intestinal tract (primarily the large intestine), and genital/urinary tract tend to harbor the most microbes; however, certain species are favored in each biological niche of our body (FIG. 1.7). For example, the skin's salinity limits the types of microbes that can thrive there to those that can tolerate salt. Likewise, the low-oxygen environment in the intestinal tract limits which microbes call the gut home. However, there is still tremendous diversity of life in and on us. Our gut microbiota alone is thought to include almost 5,000 bacterial species.[10] That said, not all parts of our body house normal microbiota. For example, the brain and spinal cord of healthy individuals are both microbe-free zones. (The normal microbiota of each body system is discussed in Chapters 16–21.)

What makes microbiota "normal" sometimes has to do with the location of the microbe rather than the species itself. For example, our normal microbiota often includes pathogens—27 percent of adults asymptomatically carry *Staphylococcus aureus* on their skin. However, this bacterium can cause skin infections by entering a cut, or food poisoning when toxins made by certain strains are ingested. It can even cause life-threatening sepsis if it enters the bloodstream. Fortunately, the majority of normal microbiota is harmless and tends to protect us by "crowding out" potential pathogens that might otherwise grow on or

[9] Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. Preprint on *bioRxiv*. Online only; doi: http://dx.doi.org/10.1101/036103.

[10] Almeida, A., Nayfach, S., Boland, M., et al. (2021). A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nature Biotechnology*, 39, 105–114.



Streptococcus cremoris, SEM can be found as part of the normal microbiota.

TRAINING TOMORROW'S HEALTH TEAM

Probiotics and Health

Probiotics are defined as microorganisms that provide a health benefit when ingested. In the past few decades, nutritional supplements containing living bacteria such as *Lactobacillus* and *Bifidobacterium* have become popular in the United States. These organisms can be swallowed in pill form or added to foods like yogurt or even baby formula in a powder form. Studies indicate that certain probiotics are helpful in combating or even preventing some forms of diarrhea (especially antibiotic-induced diarrhea), vaginal yeast infections, and urinary tract infections. There is also data to suggest that probiotics may help ease eczema and irritable bowel disease. There is still much research to be done, but the outlook is promising.

QUESTION 1.2

Why do you think probiotics may prevent or alleviate antibiotic-associated diarrhea?

within us. Our understanding of how normal microbiota impacts health and disease is just emerging.

Initial data suggests that some people's microbiome profile may increase the chance of certain chronic diseases or disorders, whereas other profiles may be protective. We have a long way to go in exploring potential links between the microbiome and human physiology, but a better understanding of these links may eventually lead to retooling of normal microbiota to treat certain diseases.

Establishing Normal Microbiota Babies are colonized in their first days of life by the microbes they encounter during childbirth and through early interactions with their environment and caregivers. However, data suggests that microbes may start to colonize us even before birth. Some researchers have found that the placenta, an organ unique to pregnancy that transfers oxygen, nutrients, and certain antibodies from mother to fetus, may harbor low levels of microbes, although the existence of a true placental microbiome remains debatable.[11] While additional research still needs to be performed, some studies have documented that the bacterial profile of a baby's first stool (meconium) resembles bacterial profiles documented for amniotic fluid.[12] Also, bacteria have been isolated from fetal lung samples, further suggesting that the womb may not be sterile after all.[13]

Regardless of when initial colonization by normal microbiota takes place, it is well established that our normal microbiota expands and continues to develop throughout the early weeks of life and evolves as we approach adulthood. The developing normal microbiota of an infant is greatly influenced by whether the baby is born by cesarean section or vaginal birth, as well as by the choice of breast milk or formula for infant feeding.

Disruptions in Normal Microbiota When our normal microbiota is perturbed, our risk for certain infections may increase. A common disruption to normal microbiota is antibiotic therapy, which may kill many types of benign resident bacteria along with the pathogen being targeted. With normal microbiota reduced, opportunistic pathogens are more likely to thrive and establish infections. A common example of this is when a woman takes antibiotics to treat a urinary tract infection (UTI), only to develop a vaginal yeast infection (vulvovaginal candidiasis) soon after the antibiotic therapy concludes. The yeast *Candida albicans* is an opportunistic pathogen that is usually present in the vagina. Its growth is normally kept in check by vaginal microbiota—especially *Lactobacillus* bacteria, which make lactic acid that keeps the vaginal pH low. As antibiotics kill off these beneficial bacteria, the vaginal pH increases to a level that allows *Candida albicans* to thrive and cause vulvovaginal candidiasis. Similarly, diarrhea is a common side effect of antibiotic therapies because they disrupt the gut microbiome. Hormonal changes, diet, age, and our general environment also contribute to shifts in our normal microbiota.

Transient Microbiota Some microbes are just temporary passengers that do not persist as stable residents of our bodies. These **transient microbiota** (or *transient flora*) can be picked up through a handshake or contact with environmental surfaces. Most acquired pathogens are transient microbiota. Unlike normal microbiota, transient microbiota can be removed through hygiene—especially via proper hand-washing technique.

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[11] Kuperman, A. A., Zimmerman, A., Hamadia, S., et al. (2020). Deep microbial analysis of multiple placentas shows no evidence for a placental microbiome. *BJOG: An International Journal of Obstetrics & Gynaecology*, *127*(2), 159–169.
and
Sterpu, I., Fransson, E., Hugerth, L. W., et al. (2021). No evidence for a placental microbiome in human pregnancies at term. *American Journal of Obstetrics and Gynecology*, *224*(4), 296.e1–296.e23.

[12] He, Q., Kwok, L. Y., Xi, X., et al. (2020). The meconium microbiota shares more features with the amniotic fluid microbiota than the maternal fecal and vaginal microbiota. *Gut Microbes*, *12*(1), 1794266.

[13] Al Alam, D., Danopoulos, S., Grubbs, B., et al. (2020). Human fetal lungs harbor a microbiome signature. *American Journal of Respiratory and Critical Care Medicine*, *201*(8), 1002–1006.

Biofilms

Biofilms are sticky communities made up of single or diverse microbial species (FIG. 1.8). They allow microbes to coordinate responses within an environment, making the community much more durable than single, **planktonic** (free-floating) bacteria. The cells that seed a biofilm often have adhesion factors such as fimbriae (short, hair-like projections discussed in Chapter 3) to help them attach to a target surface. They then secrete a sticky substance that forms a protective matrix in which the bacteria grow. Multiple layers tend to develop in the biofilm as populations expand, with the residents of the innermost layers being highly protected. Periodically, microbes in the film are released as free-growing planktonic cells, allowing them to spread to other areas and serve as a source for chronic infection.

Dental plaque is an example of a biofilm. Each day we brush our teeth to remove it and prevent dental caries (cavities) from forming, but it persists on teeth despite brushing, so the plaque quickly grows back. Biofilms can develop on nearly any surface, including contact lenses, water filtering units, cutting boards, and catheters. The National Institutes of Health estimate that between 60 and 80 percent of infectious diseases in humans are due to biofilm-creating microbes. Whereas dental plaque can be scrubbed off regularly to limit cavities, internal biofilms are not so readily managed. They are more resistant to antibiotic treatments and more difficult for our immune system to destroy than planktonic bacteria. Numerous conditions, including chronic lung infections in cystic fibrosis patients, kidney stones, inner ear infections, atherosclerosis, endocarditis, and urinary tract infections involve biofilms. Scientists are working to understand how bacteria establish them and what allows them to persist, in the hope of creating better treatments against them. (See more on biofilms in Chapter 5.)

Environmental and Industrial Uses for Microbes

It is well known that environmental toxins affect human health. The Environmental Protection Agency (EPA) documents thousands of chemical spills per year in the United States alone. **Bioremediation** harnesses the power of microbes to help clean up toxic waste. In bioremediation, certain nonpathogenic microbes are used to metabolize toxic substances into harmless intermediates. For example, hundreds of bacteria, archaea, and fungi species degrade petroleum oil spills into carbon dioxide. Bioremediation typically involves introducing nitrogen, sulfur, phosphate, and sometimes iron supplements to a spill environment along with dispersants, which break oil into smaller droplets to encourage microbes to more effectively break down the oil slick.

No cure is without a cost, and even bioremediation has its downsides, such as disturbing the microbe balance in the area, which can decrease the dissolved oxygen levels of the local aquatic environment and adversely impact the health of other organisms. The dispersants used to aid bioremediation might also harm the environment. There is no easy or obvious answer during environmental disasters, and ultimately, decisions come down to a cost/benefit analysis that tries to pick the course of action that will do the least harm. Oil slicks are not the only hazards to consider: Arsenic, mercury, and selenium are also dangerous contaminants that, in part, can be removed from contaminated soil and water through bioremediation.

In addition to cleaning up our environment, microbes have major roles in other industries. Bacteria and yeast serve as key ingredients in recipes for vinegar, beer, wine, chocolate, cheese, sauerkraut, kimchee, yogurt, sour cream, buttermilk, and bread. We tap microbes to help us produce drugs, including bacitracin, erythromycin, penicillin, and streptomycin. Microbes make important precursors for consumer products and are a source for biodegradable

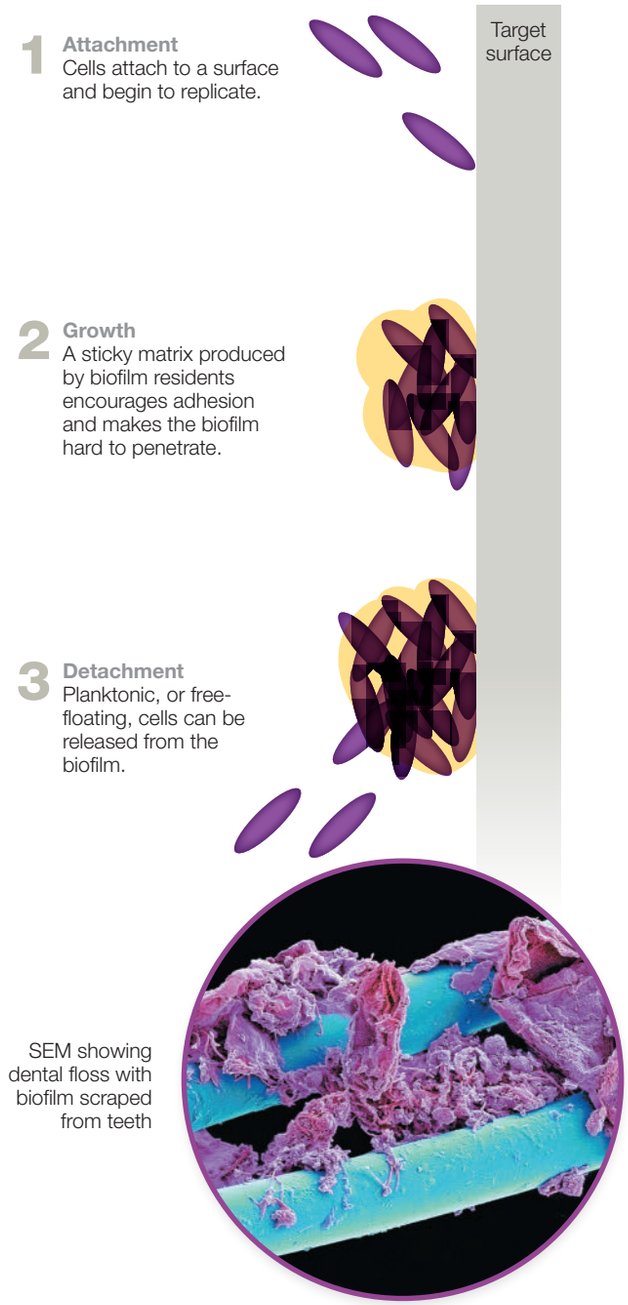

FIGURE 1.8 Biofilms Microbes form biofilm communities. Biofilms are medically concerning because they are difficult to treat, can affect medical implants and devices, and can serve as a chronic source of infection.

Critical Thinking *Drug doses sufficient to kill planktonic cells may be ineffective against biofilm microbes. Explain why this makes sense.*

plastics. For example, *Xanthomonas campestris* (ZAN-tho-moe-nas kam-PES-tris) bacteria make a natural polysaccharide commonly called xanthan gum, which is used in a variety of cosmetics and foods as a thickening agent. Microbes are even a potential source for biofuels—researchers are working to engineer *E. coli* that can metabolize sewage to produce diesel fuel.

Build Your Foundation

9. List the taxonomic rankings in order from the broadest classification to the most specific. (LO 1.8)
10. What is a strain? (LO 1.9)
11. Explain the parts of a scientific name and the basic rules for writing one. (LO 1.10)
12. Compare and contrast parasitism, commensalism, and mutualism. (LO 1.11)
13. Describe the term *normal microbiota*. Give examples of how it could be disrupted, and discuss why disruption of normal microbiota is medically concerning. (LO 1.12)
14. Why has the sickle cell trait been perpetuated in populations exposed to malaria? (LO 1.13)
15. What are biofilms, and why are they a medical concern? (LO 1.14)
16. What are the pros and cons of bioremediation? (LO 1.15)
17. List three industrial uses for microbes. (LO 1.15)

Build Your Foundation (BYF) Quick Quiz: Visit the **Mastering Microbiology** Study Area to quiz yourself.

1.3 GROWING, STAINING, AND VIEWING MICROBES

Learning Outcomes

After reading this section, you should be able to:

1.16 Discuss the main formats of culture media used in the laboratory.
1.17 Describe the goal of aseptic culture technique, and note elements that are central to it.
1.18 Explain the goal of the streak plate technique, and state why it is important in microbiology.
1.19 Summarize simple versus structural staining techniques, and discuss what information they provide about a sample.
1.20 Describe the Gram stain procedure, why it works, and sources of error in the procedure.
1.21 Explain how the acid-fast stain is performed, why it works, and its clinical uses.
1.22 Correctly label the parts of the compound light microscope, and describe the general features of bright field microscopy.
1.23 Define the term *resolution*, and describe how immersion oil and wavelength impact it.
1.24 Compare and contrast transmission electron, scanning electron, and fluorescence microscopy.

We culture microbes so we can study them.

The first step in studying a microbe is to try to grow it in the laboratory setting. This is easier said than done for the majority of known species. Unfortunately, microbes often require complex growth environments that are poorly understood, or perhaps not replicable with today's culturing techniques.

Introduction to Growth Media

Growth media or **culture media** (singular: *medium*) are mixtures of nutrients that support organismal growth in an artificial setting. Robert Koch, the scientist known for his disease postulates, assembled the first research team that used agar, a polysaccharide from seaweed, as a growth medium. Agar proved to be an ideal solidifying agent for bacterial culture, allowing for isolation and identification of individual species in a way that earlier nutrient mixtures in broth or gelatin form had not. A research associate of Koch's, Julius Richard Petri, later developed the petri dish, which when filled with the agar-solidified media, made it even easier to isolate and observe bacteria.

Media come in a wide variety of consistencies and formulations (recipes), the most common of which are nutrient agar and nutrient broth. There are also a variety of formats: Most culture media are poured as plates (a petri dish filled with solidified medium), deeps, slants, or broths (**FIG. 1.9**). Each type of media format has its advantages and disadvantages. Choosing the appropriate recipe and media format depends on the goal at hand. (Types of media are further explored in Chapter 7.)

Aseptic Culture Techniques

In nature, microbes do not tend to grow in **pure culture**—that is, they rarely exist as single-species groups. As such, it is often desirable to isolate a specific type of microbe from a diverse multitude in a collected sample. Characterizing cultures depends on **aseptic culturing techniques**, where conditions are maintained to limit contaminants, so that only the desired microbes in a given sample are grown. As a part of aseptic culture technique, the media used to grow the specimen is sterile, as are all the instruments and lab ware (tubes, plates, inoculation instruments) that directly touch specimens. (Sterilization methods are covered in Chapter 7.) Commonly, surrounding surfaces are also decontaminated before and after handling cultures; gloves and other protective clothing may also be required depending on the specimen being studied.

In some labs, samples are handled in a biological safety cabinet to minimize the chances of contaminating the culture and also to protect the researcher. A **biological safety cabinet** is a large piece of equipment that maintains a specific flow of filtered air and also is readily decontaminated using UV light and surface cleaning with an antimicrobial solution (FIG. 1.10).

The **streak plate technique** helps to isolate a specific species of microbe for study. The general goal is to spread the

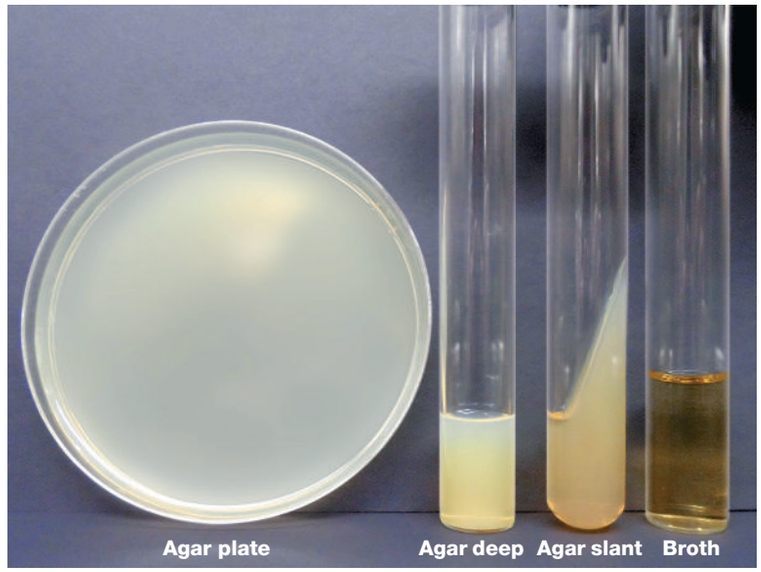

FIGURE 1.9 Culture media Culture media are typically poured as plates, deeps, slants, or broths. An agar plate, which is a petri dish filled with agar-solidified media, is used to isolate bacterial species. Culture media can be poured into test tubes, and agar can be added to the recipe to prepare solidified deeps and slants, or used without solidifying agar as a liquid broth.

FIGURE 1.10 Safety in the lab Biological safety cabinets are used to practice aseptic culturing techniques of certain samples. UV lights to decontaminate the air and surfaces in the cabinet, along with controlled airflow, make biosafety cabinets an ideal way to limit sample contamination while also protecting the researcher.

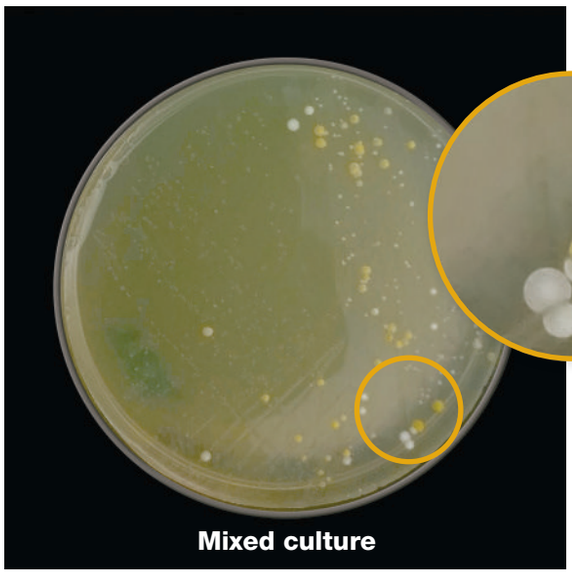

FIGURE 1.11 Isolating colonies The streak plate technique allows for isolation and characterization of individual colonies. *Top*: The goal of the streak plate technique is to spread the sample out thinly enough so that individual cells are isolated on the surface of the agar-solidified media. After an incubation period, colonies are visible on the plate. Each individual colony has developed from a single cell. *Bottom*: You can see from the variety of colonies on this plate that this sample is a mixed culture, not a pure culture.

CHEM • NOTE

The **pH scale** describes how acidic or basic a substance is based on hydrogen ion (H^+) concentration. A pH of 7 is neutral; above 7 is basic; below 7 is acidic.

sample thinly enough on an agar plate so that the various cells in the sample are sufficiently separated and can give rise to individual colonies (FIG. 1.11, *top*). A **colony** is a grouping of cells that developed from a single parent cell. The cells in a colony are genetically identical to the parent cell; they are clones. The characteristics of the colonies on a plate can be helpful for identifying a microbial species. For example, some bacteria grow in different colors, and some have characteristic margins to their colonies. When grown on a plate, a pure culture has colonies that appear somewhat uniform in shape and color, even when the colonies vary in size. By contrast, a plate with a **mixed culture** will have at least two characteristically different colonies (FIG. 1.11, *bottom*). (Additional culture techniques are reviewed in Chapter 7.)

Specimens are often stained before viewing with a microscope.

Imagine trying to find a clear marble at the bottom of a deep swimming pool—it would be a challenging task. However, if the marble were colored, it would be much easier. The same is true of specimens viewed under the microscope. The very first **stains**, or dyes, used in microbiology were added simply to increase contrast so the sample was easier to see. Eventually certain stains became an integral part of classifying microbes.

Most bacterial staining techniques involve first making a **smear** of the specimen, which involves placing a small amount of the sample on a glass slide. In most staining techniques the smear is then **fixed** by exposing it to heat or by adding a chemical reagent. Fixation adheres the sample to the slide, so that it is not as easily washed away during the staining process, and it kills most of the cells on the slide, making the stained specimen safer to handle.

After fixation, the specimen is stained. Dyes used are typically organic molecules with distinct coloration. The most common ones are sometimes called **basic dyes** because they are mildly basic (alkaline) on the pH scale. Also, these dyes are positively charged—therefore, the stain is attracted to the negatively charged cell surface of microbes and easily enters the cells. Frequently used basic dyes include methylene blue, crystal violet, safranin, and malachite green. Occasionally, **acidic dyes** such as nigrosin or India ink are also used. Acidic dyes are negatively charged, so they do not easily enter cells. Instead, they stain the background of a specimen in a technique called **negative staining**. An advantage of negative staining is that it doesn't require heating or chemical fixation, and the dye is not absorbed by the sample. This means the sample has a more true-to-life appearance, with fewer distortions of delicate cellular features. When two dyes are used in the same staining procedure, the first dye is often called the primary dye and the second dye is often called the **counterstain**.

Mordants are chemicals that may be required in certain staining procedures to interact with a dye and fix, or trap, it on or inside a treated specimen. There are numerous types of mordants such as iodine, alum, and tannic acid. Microbiologists use many staining techniques, but most can be classified as either simple, structural, or differential.

Simple Stains

Simple staining techniques use one dye. *Typically only size, shape, and cellular arrangement can be determined using simple stains.* A common protocol involves covering a heat-fixed smear with a basic dye such as methylene blue for about a minute, so that the dye has enough time to penetrate the cells. Then the smear is gently rinsed with distilled or deionized water to remove excess dye. A cover slip is placed over the smear, and it is ready for viewing with a light microscope (FIG. 1.12).

Structural Stains

Many microbes have specialized structures that can be seen using staining techniques that bring out these structural features so they may be easily viewed under a microscope.

Flagella Staining Some cells use whip-like extensions called **flagella** for motility. While eukaryotic microbes tend to have only one flagellum located at a single pole of the cell, prokaryotes can have single or multiple flagella with diverse arrangements. The number and position of flagella can help identify the microbe, making staining techniques to view flagella useful. Because flagella are very thin and difficult to see, staining methods coat samples with chemicals that act as mordants (substances that help dyes stick to a target) and also use dyes designed to thicken the appearance of flagella for easier viewing (**FIG. 1.13**, *left*).

Capsule Staining **Capsules** are sticky carbohydrate-based structures that some bacteria produce as a form of protection and also to help them adhere to surfaces. Certain pathogens require a capsule to establish an infection. Because capsules are easily dissolved in water and do not readily take up basic dyes, a negative staining technique utilizing an acidic dye is usually used in coordination with a basic dye. The basic dye stains the actual cell, while the acidic dye stains the background to enhance contrast. A capsule then appears as a clear halo around a stained cell (**FIG. 1.13**, *middle*).

Bacterial Endospore Staining Certain bacteria, including *Bacillus* species such as *B. anthracis*, make specialized dormant structures called **endospores** in response to stressful or harsh environmental conditions. Very few bacteria form them, so detecting endospore presence is useful for classification. Because endospores have a tough spore coat that resists staining, the technique involves heating the specimen to drive a dye, usually malachite green, into the spores. The excess dye is rinsed off, and a counterstain, usually safranin, is added to make it easier to see cellular material that surrounds the spore, or nonsporulating cells (those that do not form spores). As a result, endospores appear green, and other cellular features appear pink (**FIG. 1.13**, *right*). (Endospore formation and characteristics are reviewed in Chapter 3.)

Differential Stains: Gram and Acid-Fast

Differential staining highlights differences in bacterial cell walls, anatomical structures that contribute to cell rigidity. (Bacterial cell wall structure and function are discussed further in Chapter 3.) These differences in cell-wall structure

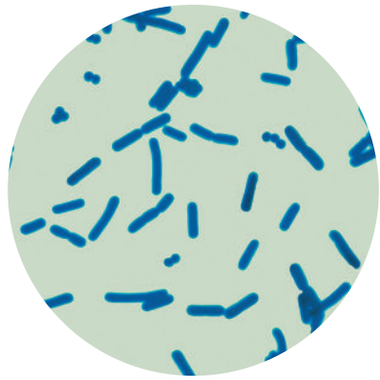

FIGURE 1.12 Simple stain Methylene blue stain of *E. coli* reveals small rods with a single arrangement.

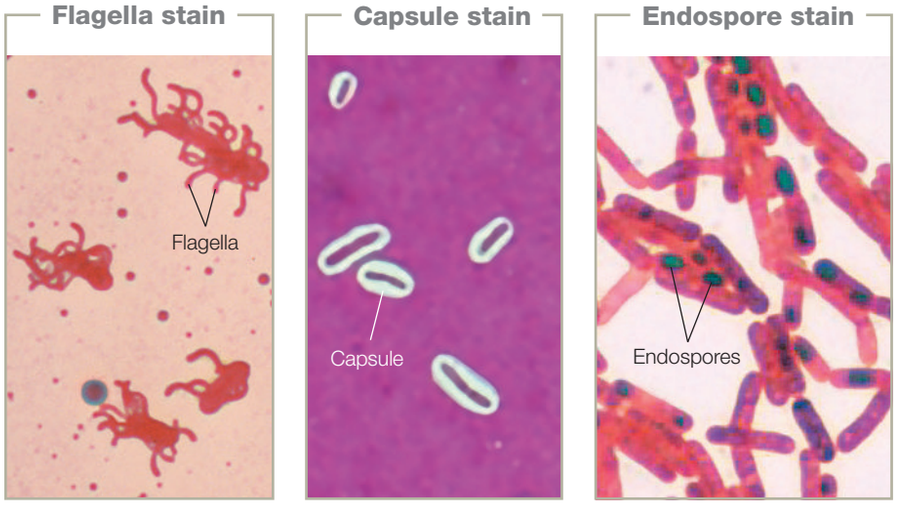

FIGURE 1.13 Structural stains Flagella staining reveals flagella at each pole of this bacterial cell. Capsule staining shows a clear halo surrounding pink-staining bacterial cells. Endospores appear green following the endospore stain.

Critical Thinking *Technically, any basic dye could be used as a secondary stain in the endospore stain, so why is pink (safranin) an ideal choice?*

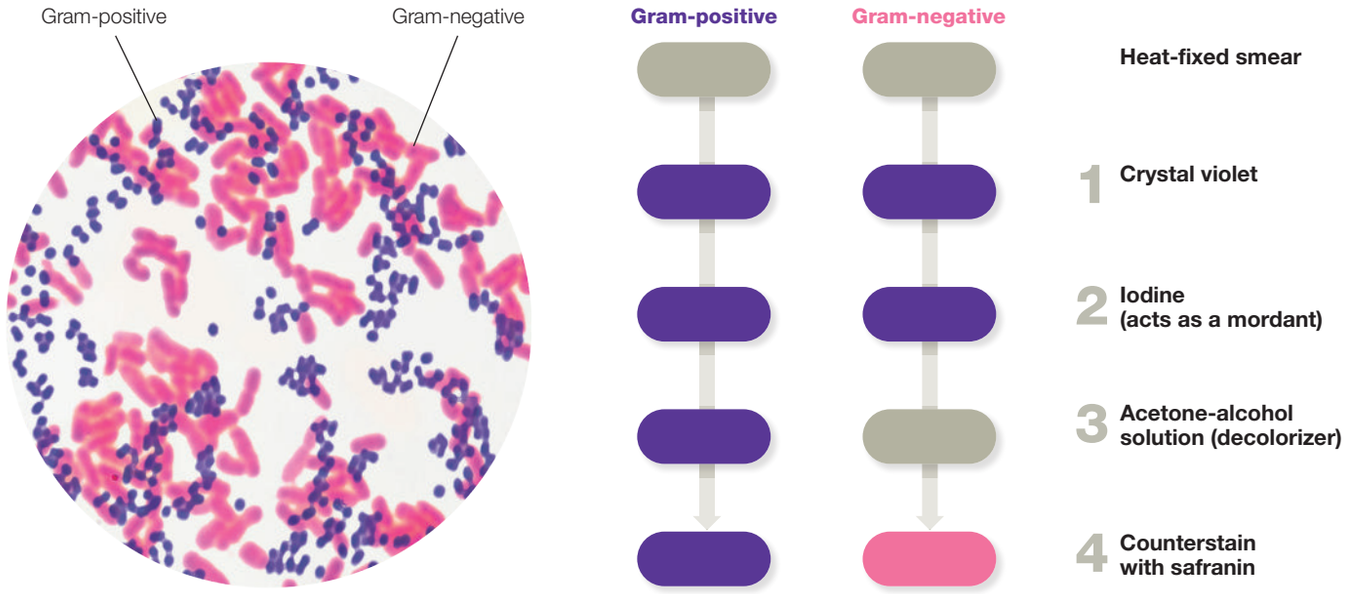

FIGURE 1.14 Gram staining *Left*: Gram-positive bacteria appear purple at the end of the Gram staining procedure while Gram-negative bacteria appear pink. *Right*: Summary of the Gram stain; notice that Gram-positive bacteria remain purple throughout the procedure, whereas Gram-negative bacteria are decolorized upon addition of the decolorizing solution, and then counterstained pink.

Critical Thinking *What color would Gram-positive bacteria be if the iodine step was omitted?*

Gram Stain

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are clinically significant. The two most common differential stains used in microbiology are the Gram stain and the acid-fast stain.

Gram Staining The **Gram stain** is one of the most important stains in microbiology. This process classifies bacteria as either Gram-positive or Gram-negative. The Gram property of a specimen has important clinical implications, including the potential pathogenic features of the organism, and what antibiotics might be most effective in combating it. For example, penicillin is generally more effective at combating Gram-positive bacteria than Gram-negative bacteria.[14] Polymyxin antibiotics are typically effective against Gram-negative bacteria, but will not control infections caused by Gram-positive bacteria. Although bacterial cell wall structure isn't the only feature that influences whether a bacterium will be susceptible to a given drug, it is a key factor for defining general susceptibilities to certain classes of drugs.

The Gram stain works by revealing chemical and structural features of cell walls. It is important that you understand that the Gram stain procedure simply highlights differences in cell wall structures; it does *not* chemically turn a bacterium into a Gram-positive or Gram-negative specimen, any more than a pregnancy test makes a woman pregnant. *By the end of the Gram stain, Gram-positive cells appear purple, while Gram-negative cells appear pink* (FIG. 1.14).

The Gram stain technique is as follows:

1. A heat-fixed bacterial smear is stained with **crystal violet**, giving all the cells a deep purple color. This step is called the *primary stain*.
2. After briefly rinsing with water to remove excess crystal violet dye, an **iodine** solution is added to the sample. This acts as a mordant, forming an insoluble crystal violet-iodine complex (CV-I complex). At this point all cells remain purple.

[14] Not all Gram-positive bacteria are susceptible to penicillin family drugs. Some acquire drug-resistance factors such as the ability to break it down. In contrast, Gram-negative bacteria are naturally resistant to certain penicillin family drugs because they tend to struggle to get past the outer membrane structure. Because Gram-positive bacteria lack an outer membrane, barring the acquisition of resistance factors, they will generally respond well to penicillin-based drugs.

3. The sample is briefly rinsed with an **acetone-alcohol** solution. Although this is known as the *decolorizing step*, Gram-positive bacteria remain purple; only Gram-negative bacteria are decolorized and thereby left colorless after this step.
4. Because the prior step left Gram-negative bacteria colorless and hard to see on a glass slide, the last step of the Gram stain involves covering the smear with **safranin**, a counterstain, which stains the decolorized cells pink. Even if you know the Gram property, all samples are subjected to step 4. Before the sample is viewed under the microscope, the excess safranin is gently rinsed away with water, the slide is allowed to dry, and a cover slip is adhered to the slide.

Although Hans Gram developed the technique in the late 1800s, the mechanism for how the staining technique distinguishes between Gram-positive and Gram-negative bacteria (during decolorization, step 3) was not understood until the 1980s. What occurs in the different cell types has to do with cell wall composition.

Gram-positive bacterial cell walls contain a thick layer of a protein-carbohydrate substance called **peptidoglycan**, whereas Gram-negative bacterial cell walls have only a thin peptidoglycan layer. Additionally, Gram-positive bacteria do not have an outer membrane, whereas Gram-negative bacteria have one lying just on top of their thin peptidoglycan layer. This outer membrane is important to detect because it contains **lipopolysaccharide (LPS)**, of which the lipid portion is toxic to animals and can trigger septic shock. Although the outer membrane is rich in lipids, it is vulnerable. During the few seconds that the acetone-alcohol is in contact with Gram-negative bacterial cells, it dissolves the outer membrane of the cell wall to expose the thin peptidoglycan layer. The chemistry of this step renders the Gram-negative cell wall leaky, such that the purple CV-I complex formed during step 2 easily washes out of the Gram-negative bacteria. In contrast, Gram-positive bacteria remain purple because the acetone-alcohol solution dehydrates the thick peptidoglycan layer causing it to pack down and trap the CV-I complex inside Gram-positive cells.

The trickiest part of the Gram stain is the decolorizing step (step 3). If you leave the decolorizer on a sample too long, then even the thick peptidoglycan layer of Gram-positive cell walls will be damaged to the point of becoming leaky, allowing the CV-I complex to be readily rinsed out of the cells. Thus, over-decolorizing a sample leads to inaccurate results in which all cells appear pink irrespective of their true Gram property.

Other things can make interpreting results difficult, even when the procedure is done correctly. For example, *Acinetobacter* species have cell walls that are especially resistant to the action of the decolorizing solution, so these bacteria may appear Gram-positive using standard Gram staining methods despite the fact they are actually Gram-negative bacteria.[15] Another example of unexpected Gram staining result is found in *Mycobacterium* species, which include pathogens that cause tuberculosis and leprosy. These Gram-positive bacteria have a waxy cell wall that resists staining with crystal violet, making them appear Gram-negative.[16] Other bacteria appear to consist of both Gram-positive and Gram-negative cells, depending upon the life stage of the cells. For example, sporulating bacteria such as *Bacillus* species, which include *B. anthracis*, the cause of anthrax, and *Clostridium* species[17], which include the pathogen

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In the Gram Scheme of Things

One example of the vital role of microbiology labs in patient care is the diagnosis and treatment of bacteremia—a bacterial infection in the bloodstream. These infections have a mortality rate of about 20 percent in hospitalized patients. The first indication of bacteremia is usually based on Gram staining data for a blood sample. Gram-positive or Gram-negative bacteria can cause bacteremia, but knowing which is the culprit is important, as the Gram property influences the choice of antibiotic used.

When a physician receives the microbiology lab report, the empirical treatment (the treatment initiated prior to a definitive diagnosis) is often changed based on the Gram property data received.

QUESTION 1.3

Most Gram typing errors involve Clostridium *and* Bacillus *species. Based on your readings, why are these genera of bacteria particularly problematic in terms of Gram typing accuracy?*

CLINICAL VOCABULARY

Septic Shock A serious complication of a system-wide infection that can lead to organ failure and death. This complication is often, though not exclusively, associated with elevated levels of LPS that Gram-negative bacteria make. Signs and symptoms are usually nonspecific and can include fever, chills, nausea, low blood pressure, and difficulty breathing.

[15] Bazzi, A. M., Al-Tawfiq, J. A., & Rabaan, A. A. (2017). Misinterpretation of Gram stain from the stationary growth phase of positive blood cultures for Brucella and Acinetobacter species. *The Open Microbiology Journal*, *11*, 126.

[16] Kuroda, H., & Hosokawa, N. (2019). Gram-ghost bacilli. *Journal of General and Family Medicine*, *20*(1), 31–32.

[17] *C. difficile*, a bacterium that cause pseudomembranous colitis, also forms endospores.This bacterium was fairly recently shifted from the genus *Clostriduium* and reclassified into the genus *Clostridioides*. As seen for *Bacillus* and *Clostridium*, sporulating bacteria in the genus *Clostridioides* also exhibit variability in Gram stain results.

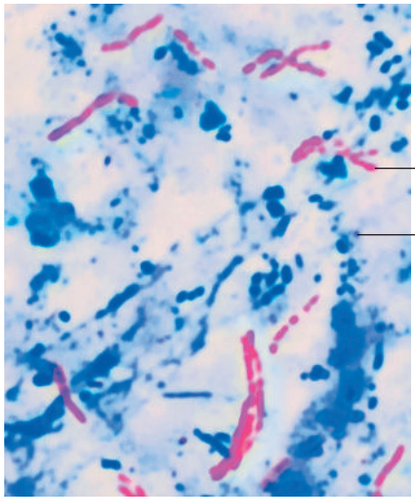

FIGURE 1.15 Acid-fast stain The most common acid-fast staining procedure is the Ziehl–Neelsen method. Acid-fast bacteria appear pink, and non–acid-fast bacteria are blue.

Critical Thinking *What color would non–acid-fast bacteria be if the decolorizing step with acid-alcohol was omitted?*

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responsible for botulism, appear Gram-negative when cells are forming endospores. But those same cells appear Gram-positive (their true classification) while healthy and nonsporulating.

To minimize Gram property errors, fresh cultures between 24 and 48 hours old should be used, so that the cells tested are mostly healthy and undamaged. The older the sample cells, the more variable the Gram results may be. It is also a good practice to include a positive control (something you know is Gram-positive) on all test slides to ensure that the procedure is being properly performed.

Acid-Fast Staining The **acid-fast stain** is a differential stain that distinguishes between cells with and without waxy cell walls. Acid-fast bacteria have waxy cell walls that are rich in a substance called **mycolic acid**; they retain the primary dye, which is red, even after exposed to an acid wash. In non–acid-fast cells, the acid wash strips away the red stain (the primary stain). Clinically, the acid-fast stain is an important diagnostic tool for detecting *Mycobacterium* species such as the causative agents of tuberculosis and leprosy. The acid-fast stain also detects *Nocardia* species, bacteria that are usually in soil and water and can act as opportunistic pathogens of immune-compromised patients.

There are several acid-fast staining procedures, but the most common method is the Ziehl–Neelsen (Zeel NEEL-sen) method, where the primary dye, *carbol-fuchsin* (CAR-bowl-FEWK-sin), is layered onto a heat-fixed smear, and the sample is steamed for several minutes to drive the red dye into the bacteria. After a thorough water rinse, the sample is then briefly treated with an acid-alcohol solution, which acts as a decolorizing agent. This is the differentiating step; overdecolorizing with the acid-alcohol rinse can lead to false-negative results. Finally, a counterstain, *methylene blue*, is added. Because acid-fast bacteria have a waxy cell wall that resists decolorization by the acid-alcohol rinse, they appear a bright pink-red at the end of the procedure. Non–acid-fast bacteria appear blue at the end of the procedure (FIG. 1.15).

Microscopy is central to microbiology.

Most early microscopes only magnified a specimen about 10 times (10×), which is not powerful enough to see most bacteria. Van Leeuwenhoek's microscopes of the 1600s reached about a 300× magnification, allowing him to see and describe basic bacterial shapes and arrangements. Today's microscopes allow us to see substances that are 20 million times smaller than the visibility threshold of the naked eye. Our technology allows us to glimpse viruses, most of which are much smaller than most bacteria. We can also visualize atoms and even the bonds between atoms. **Micrographs**, or pictures taken through a microscope, allow us to document and share microscopy observations.

Light Microscopy

Light microscopy uses visible light to illuminate the specimen. This is one of the simpler ways to view a sample. As photons in a light wave interact with the specimen, they are channeled up to the viewer's eyes through a series of lenses. The type of lens used determines the final magnification observed. Also, the quality of the glass used to make the lens, as well as the shape of the lens itself, contribute to the quality of the final image. The **compound light microscope** is the most common type of optical microscope and is a basic tool found in microbiology labs (FIG. 1.16).

Parts of the Compound Light Microscope The **objective lens** is near the specimen, whereas the **ocular lens** sits at the top of the microscope near the viewer's eyes. The final magnification of the specimen is determined by multiplying the magnification of the ocular and objective lenses. For example, if the ocular lens has a 10 × magnification and the objective lens provides 40 × magnification, then the final magnification is 400 × (10 × 40 = 400 ×). Most ocular lenses

magnify a specimen by 10×, though some are 15×. Objective lenses come in varieties that usually include 4×,10×,40× and 100×.

Light microscopes also depend on a **condenser**, consisting of lenses that sharpen light into a precise cone to illuminate the specimen. The condenser also has an **iris diaphragm** that allows the viewer to modulate how much light is aimed at the specimen in order to improve contrast. The light that interacts with the specimen is channeled through the objective lens, which magnifies the image and also enhances the resolution. After the light passes through the objective lens, it continues up to the ocular lens, where the image is further magnified before reaching the viewer's eyes. The **coarse focus knob** on the microscope allows the viewer to roughly focus the image by affecting the distance of the objective lens from the specimen. The **fine focus knob** does the same, but in much smaller increments than the coarse knob, allowing for precision focusing.

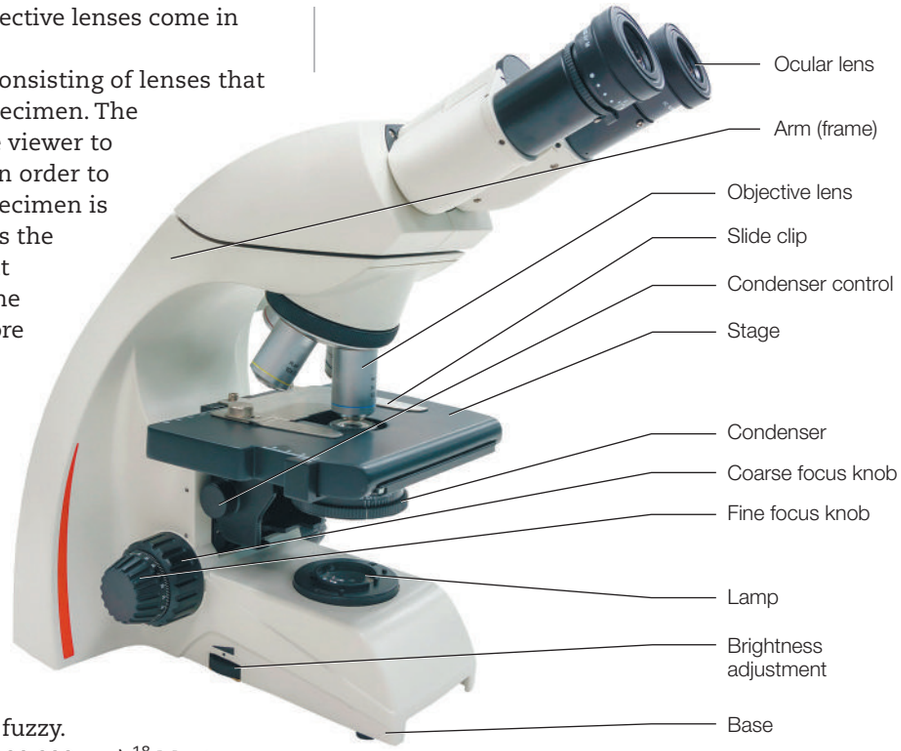


FIGURE 1.16 Parts of a compound light microscope

Resolution The ability to distinguish two distinct points as separate is **resolution**. You can think of resolution as being how sharp your vision is. A toy microscope may have decent magnification, but because its lenses are usually of low quality with poor resolving power, the images generated will be large but fuzzy.

The naked eye has a resolution of about 0.1 mm (100,000 nm).[18] Most compound light microscopes magnify a maximum of 1,000–1,500×. While greater magnification can be achieved, it wouldn't improve the image observed because the greatest resolution achievable with a light microscope is about 0.0002 mm (200 nm), or 500 times better than the resolving power of the naked eye. This limit for resolution is due to the fact that the smallest wavelength of visible light is 400 nm.

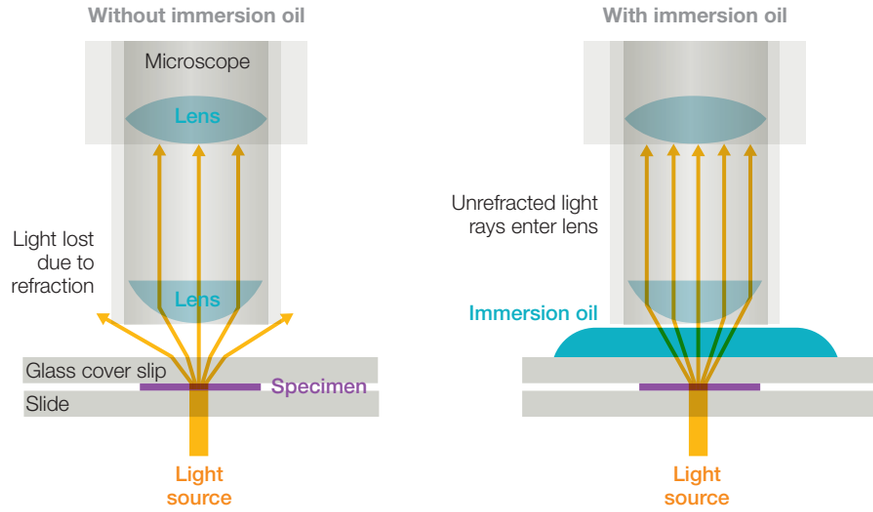
Oil immersion Another factor in the resolution of microscopes relates to **refractive index**, which is the degree to which a substance bends light. Air has a lower refractive index than glass. As such, when light passes through a slide and into the air above the slide, the light waves bend and are not channeled directly through the objective lens toward the eye. At low-power magnification, this light bending doesn't really impact resolution. However, to get a sharp image at high-power magnification (100× objective lens), **immersion oil** must be applied to the slide's surface. This specialized oil is formulated to have the same refractive index as glass. Therefore, the oil ensures that the light that interacts with the specimen on the glass slide is smoothly funneled up toward the high-power objective lens instead of scattering when it reaches the air between the slide and lens (FIG. 1.17). Oil immersion is used in a variety of light microscopy techniques—including bright field microscopy, which we'll cover next.

Bright Field Microscopy The simplest and most common form of all microscopy is **bright field microscopy**. In this light microscopy technique, a solid cone of light illuminates a sample from below, and the image is magnified through a series of lenses in a compound light microscope. The method's name, bright field, derives from the fact that the visualized sample appears as a darker contrasting image on a light background—that is, on a bright field of light. Bright field microscopy images are a product of how light is absorbed by a sample. Therefore, unless the sample has natural coloration to enhance light absorption, such as in photosynthetic cells, the specimen must be stained in

[18] One millimeter (mm) is equivalent to 1,000,000 nanometers (nm).

FIGURE 1.17 Oil immersion technique Immersion oil is used with the high-power objective lens (100×) of a light microscope to improve resolution. Because the oil has a similar refractive index to glass, light is directly channeled up through the objective lens instead of being refracted (bent) and lost to the surroundings.

Critical Thinking *Why would funneling as much light as possible through the objective lens improve resolution?*



order to be seen with bright field microscopy. Because most staining techniques kill cells, bright field microscopy is less than ideal for viewing live specimens. Dark field, phase contrast, and differential interference contrast microscopy, all of which are summarized in **TABLE 1.3**, are better for observing live samples.

TABLE 1.3 Comparing Light Microscopy Techniques: Amoeba *Proteus* Viewed with Different Light Microscopy Techniques

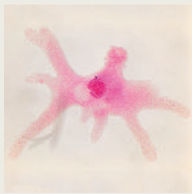
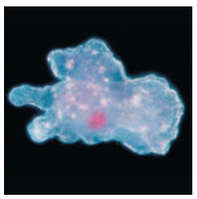
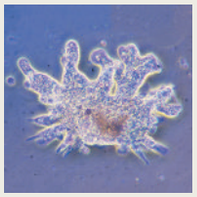
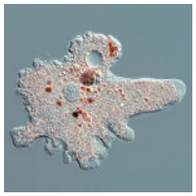
Microscopy Technique	Image	Equipment	Notes
Bright Field 	Darker contrasting image on a bright background	Compound light microscope	Illuminates sample with solid cone of light; image formed based on how light is absorbed; sample must be stained or have natural coloration
Dark Field 	Negative image, where the sample appears light on a darker background	Modified condenser in a compound light microscope	Illuminates sample with hollow cone of light; image formed based on how light is scattered as opposed to how light is absorbed, so staining is not necessary; negative image made by dark field microscopy should not be confused with negative staining; visualizes unstained specimens (live or dead) and stained specimens
Phase Contrast 	Negative image, where the sample appears light on a darker background	Modified condenser in a compound light microscope	Illuminates sample with hollow cone of light; a device in the microscope (i.e., a phase plate) interacts with light that passes through the viewed sample—thereby enhancing image brightness, shading, and contrast; visualizes unstained specimens (live or dead) and stained specimens
Differential Interference Contrast (or Nomarsky) 	One side of specimen appears brighter than the other side, providing a false three-dimensional appearance	Modified compound light microscope	Illuminates specimen with polarized light (uniformly oriented light) as opposed to a hollow cone of unorganized nonpolarized light used in phase contrast microscopes

TABLE 1.4 Comparison of Electron Microscopy to Light Microscopy

Light Microscopes	Electron Microscopes
Use light waves to image the specimen	Use an electron beam to image the specimen
Small, portable, and affordable	Large, requires special designated space, expensive
Simple, cheap, and easy sample preparation that requires minimal training	Lengthy and complex sample preparation requires substantial training
Color images possible	Only black-and-white images (though color may be added later, as an aftereffect)
Most microscopes provide a maximum of 1,000×	Can magnify over 500,000×
Resolution of 200 nm	0.2 nm or about 1,000 times better than the best compound light microscopes
Specimens can be living or dead	Specimens are all dead
Stains often used, but certain forms can be done without staining and can visualize live cells	Specimens often must be stained with an electron-dense substance like osmium or gold

Electron Microscopy

As stated before, the smallest wavelength of visible light is 400 nm. *Resolution improves with smaller wavelengths*. Because an electron beam has a much smaller wavelength of about 1 nm, it is ideal for probing ultra-small structures like viruses, which are too small to be seen with light microscopes.

There are two main classes of electron microscopes: transmission electron microscopes and scanning electron microscopes. Both shoot electrons at a specimen and then generate an image by registering how the electrons interact with the specimen. While electron microscopes provide high-magnification and high-resolution images, they are very expensive, and they require considerable training to use. A comparison between electron microscopy and light microscopy is provided in **TABLE 1.4**. Electron micrograph examples are shown in **FIG. 1.18**.

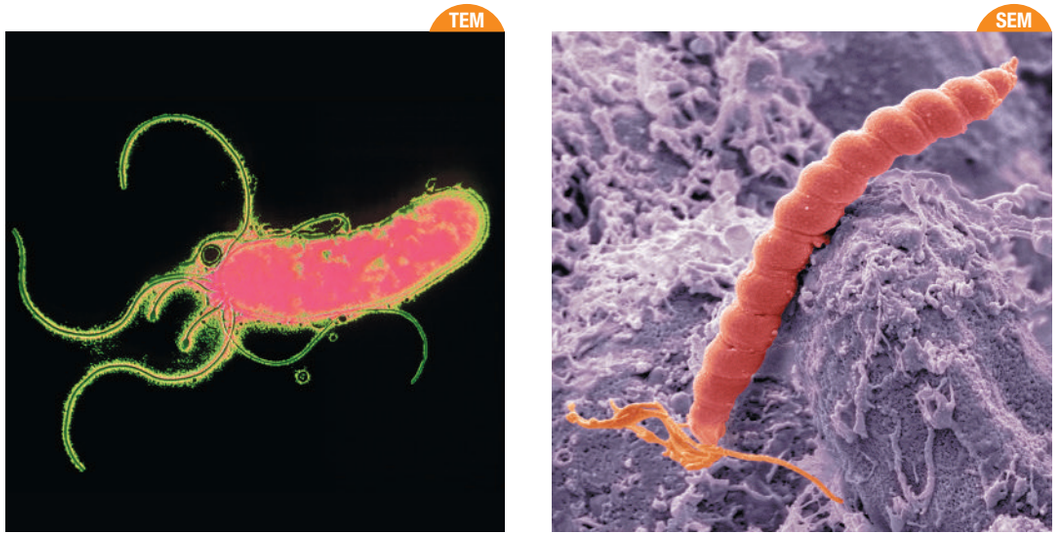


FIGURE 1.18 Examples of electron micrographs *Left*: Transmission electron micrograph (TEM) of *Helicobacter pylori*, a bacterium that causes ulcers. Note the numerous flagella. *Right*: Scanning electron micrograph (SEM) of *H. pylori* shows surface details based on how electrons are reflected off of an electron-dense sample surface. When you see colors in electron micrographs, they have been falsely generated for artistic effect.

Critical Thinking *Why are all electron micrographs naturally black-and-white images?*

Transmission Electron Microscopy (TEM) TEM is the most common form of electron microscopy. It provides up to 1 million times magnification and resolution, about 1,000 times better than that of light microscopy. Unfortunately, TEM samples must be extensively pretreated, and the maximum thickness of a specimen viewable with this type of microscope is 285 times thinner than a human hair. In TEM the electron beam passes through the specimen and hits a detector to generate two-dimensional black-and-white images of internal structures of the specimen. Dark areas represent regions where electrons were poorly transmitted, whereas light areas represent less dense regions where electrons were more readily transmitted. TEM is important to many fields, including biomedical research and nanotechnology. TEM has been central to understanding viruses and how they interact with host cells, as viruses are too small to be viewed by light microscopes.

Scanning Electron Microscopy (SEM) Hard, dry specimens like bone usually require no preparation for SEM. But soft tissues or organisms being viewed by SEM are usually preserved, dehydrated, and coated in an ultra-thin layer of conductive metal such as gold or a gold/palladium alloy. Then an electron beam scans over the specimen. Specialized detectors sense how the electrons interact with the surface of the specimen, and related signal information generates a black-and-white three-dimensional image. The overall resolution and magnification potential of SEM is lower than TEM, but SEM is useful because it provides excellent information about surface structures, whereas TEM provides information about internal structures.

SEM techniques have dramatically aided our understanding of the surface features of a variety of cells and viruses. They revealed that dramatic surface structure changes can occur in tissues with certain pathological conditions like kidney disease or cancer. SEM is also useful to study biofilms. Forensics uses SEM to examine crime scene evidence, and nanotechnology uses SEM for quality control. Certain SEM techniques can also help identify the chemical components of a substance.

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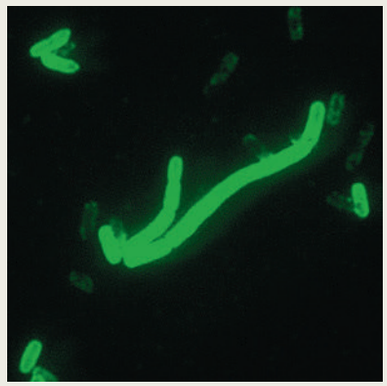
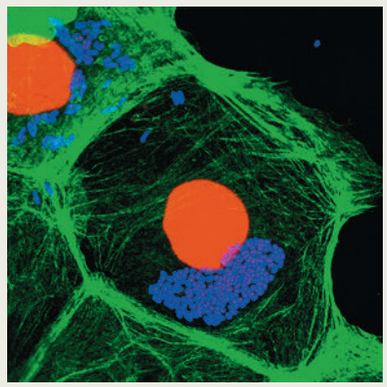
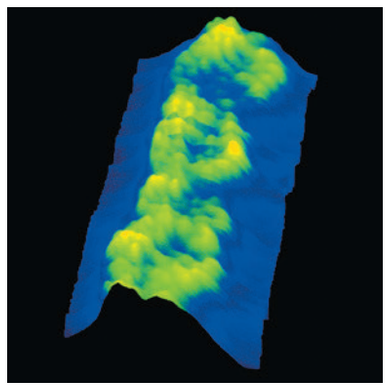
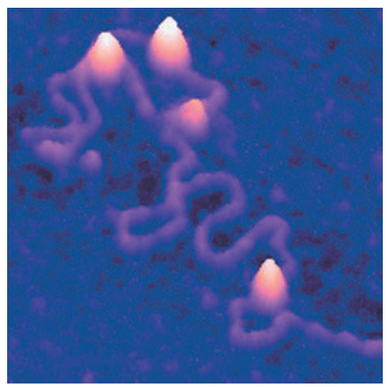
Using Fluorescence in Microscopy

Fluorescence is a naturally occurring phenomenon in which a substance absorbs energy, usually ultraviolet (UV) light that is invisible to the human eye, and then emits that energy as visible light. Many marine creatures, microbes, and minerals exhibit natural fluorescence. There are also a number of fluorescent dyes, called **fluorochromes**, which can be used to stain samples so they will fluoresce when illuminated by a UV light microscope. Fluorochromes can be natural or synthetic, and many chemically interact with certain cellular features. For example, Hoechst dyes bind to DNA and will glow blue when activated by UV light. The auramine-rhodamine fluorescent stain has a strong attraction for acid-fast bacteria and emits a distinct reddish-yellow glow when bound to its target, making it helpful for confirming the presence of mycobacterial species, such as those that cause tuberculosis. A fluorescing dye called calcofluor-white associates with cellulose and chitin found in the cell walls of fungi and other organisms and is helpful in rapid screening for yeasts and various pathogenic fungi.

Fluorescent dyes can also be linked to antibodies, immune-related proteins that help identify and target "nonself" cells such as bacteria. In **immunofluorescence**, a sample is exposed to fluorescent-tagged antibodies that recognize a specific target—perhaps a protein that is present only on the surface of certain pathogens. If the sample contains the target protein (or other molecule), then the antibodies will bind to the sample and glow when viewed under UV light. Immunofluorescence is used in many applications, such as rapid identification of bacteria in blood cultures, virus identification in patient samples, and fast screening for pathogenic bacteria in food-processing plants. **TABLE 1.5** summarizes some forms of UV-based microscopy and also explores non–light-based microscopy techniques.

TABLE 1.5 UV Light Microscopy and Probe Microscopy Techniques

	Microscopy Technique	Image	Type of Sample	Notes
Fluorescence Imaging	**General Fluorescence** 	Flat image with coloration based on fluorochrome used	Live or fixed	The UV waves cause visible light to be released from fluorochromes; advantage over light microscopy is not improved resolution or magnification, but easy and sensitive detection; allows for the detection of even a single molecule in a sample
	Confocal 	3D image	Live or fixed	Eliminates blurriness associated with standard fluorescence microscopes; images are taken at different planes of focus, and then these photo "slices" are compiled to generate a 3D image
Probe Techniques	**Scanning Tunneling** 	3D image	Atoms can be visualized; sample must conduct electricity, which limits what can be visualized	A probe sharpened to a single atom at the tip shoots electrons at the sample surface; elevations or dips in sample surface are registered to make the image; not as good as scanning electron microscopy for detecting steep rises or deep valleys in sample
	Atomic Force 	3D image	Atoms can be visualized; live samples under physiological conditions or fixed samples	Probe is dragged or tapped along specimen surface; not as good as scanning electron microscopy for detecting steep rises or deep valleys in sample

BENCH to BEDSIDE

A Possible Link between Lost Normal Microbiota and Immune System Disorders

Autoimmune disorders arise when a patient's immune system attacks the person's own tissues. There are over 100 different recognized autoimmune disorders that impact over 23 million Americans. Examples include lupus, multiple sclerosis, type I diabetes, and inflammatory bowel disease. Statistically speaking, people in developed nations are at a greater risk for autoimmune disorders than are people living in developing nations. Nobody knows precisely why this is the case, or how autoimmune diseases emerge. But the hygiene hypothesis aims to explain the matter.

The hygiene hypothesis proposes that the modern quest for a microbe-free environment may actually encourage our immune systems to malfunction. The hypothesis states that because humans coevolved with parasitic worms and other microbes all around them, these creatures may have a symbiotic role in the evolution of our immune response and general health. In particular, intestinal helminths (multicellular, parasitic roundworms and flatworms) remain common ailments in many developing nations but are rare in developed countries thanks in large part to stringent water sanitation practices and food safety regulations.

Under most circumstances, humans tolerate parasitic worms (helminths) fairly well. Various studies show that geographical areas with the highest incidence of helminthic infections tend to have the lowest rates of allergy and autoimmune diseases. Studies also demonstrate that when people migrate from areas where helminth infections are common to places where they are rare, incidence of autoimmune disease rises in subsequent generations living in the new location. Interestingly, other studies show that some genes predisposing a person to asthma are possibly linked to resistance to certain helminthic infections.[1] Based on animal and human studies, it seems that helminthic infections may moderate the activity of certain immune system cells, called regulatory T cells.[2] Immune systems that mature without the presence of helminthic infections may be more likely to "overreact," leading to inappropriate immune responses against self cells or benign foreign substances, such as pollen.

If immune system disorders can be stifled by helminths, it may be that reintroducing these worms into the body could lessen the severity of autoimmune disorders. Since 2003, over a dozen clinical trials have tested this approach. In one experiment, patients ingested the eggs of pig whipworm in an effort to alter or divert the host immune system response. Pig whipworms can't complete their life cycle in humans, so the spread of the worm to other people is unlikely, and sustained infection by the worms is only accomplished by periodically reinfecting the patient. While none of these studies has progressed to phase III, the final stage before approval for clinical use, the results have been promising in treating multiple sclerosis as well as Crohn's disease and other immune-mediated disorders.[3] That said, helminth therapy has a long way to go before it hits the clinic, but perhaps one day there will be probiotic worms to treat certain disorders linked to a hyperreactive immune response.

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[1] Jesus, T. D. S., Costa, R. D. S., Alcântara-Neves, N. M., Barreto, M. L., & Figueiredo, C. A. (2019). Variants in the CYSLTR2 are associated with asthma, atopy markers and helminths infections in the Brazilian population. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 145, 15–22.

[2] Maizels, R. M. (2020). Regulation of immunity and allergy by helminth parasites. *Allergy*, 75(3), 524–534.

[3] Ryan, S. M., Eichenberger, R. M., Ruscher, R., Giacomin, P. R., & Loukas, A. (2020). Harnessing helminth-driven immunoregulation in the search for novel therapeutic modalities. *PLoS Pathogens*, 16(5), e1008508.

Build Your Foundation

18. What format of media is recommended for isolating bacteria? (LO 1.16)
19. Why are aseptic culturing techniques important in the microbiology lab? (LO 1.17)
20. Define the term *colony*, and describe a technique that allows for colony isolation. (LO 1.18)
21. What information do simple stains reveal about a bacterial specimen? (LO 1.19)
22. What stain reveals if a bacterium forms endospores? Why would knowing if a bacterium forms endospores be helpful? (LO 1.19)
23. What is the Gram stain, how does it work, and what are potential sources of error in the procedure? (LO 1.20)
24. How is the acid-fast stain performed, and what are its clinical applications? (LO 1.21)
25. Identify the parts of the compound light microscope, and describe the general features of bright field microscopy. (LO 1.22)
26. How does immersion oil improve the high-power resolution of a light microscope? (LO 1.23)
27. Compare and contrast SEM and TEM. (LO 1.24)
28. What is fluorescence microscopy, and what are its advantages? (LO 1.24)

Build Your Foundation (BYF) Quick Quiz: Visit the **Mastering Microbiology** Study Area to quiz yourself.

Microbiology's Golden Age

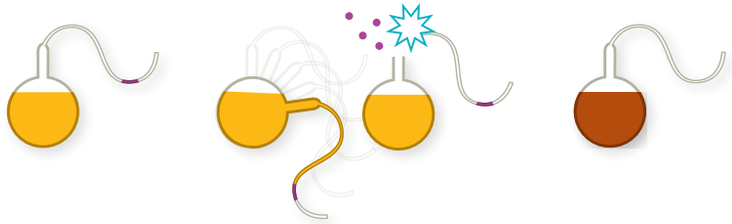
Biogenesis was accepted and the germ theory of disease embraced. Central figures were Pasteur with his S-necked flask study and Koch with his disease postulates.

Pasteur's study

1 Sterilized broth remains pure indefinitely unless...

2 ...flask is tilted, shaken, or S-neck broken...

3 ...allowing microbes to contaminate broth.



Koch's postulates

1 The same organism must be present in every case of the disease but not present in healthy individuals.

2 The organism must be isolated from the diseased host and grown as pure culture.

3 The isolated organism should cause the disease in question when it is inoculated into a susceptible host.

4 The organism must then be re-isolated from the inoculated, diseased animal.

Classifying Microbes

Organisms are classified by their shared features. A symbiotic relationship exists when two or more organisms are closely connected. Symbiotic relationships can be described as hurting the host (parasitism), helping the host (mutualism), or having no perceived benefit or cost to the host (commensalism).

CLASSIFICATION

Domain
(least specific)

Kingdom

Phylum

Class

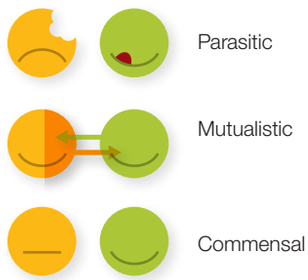
Order

Family

Genus

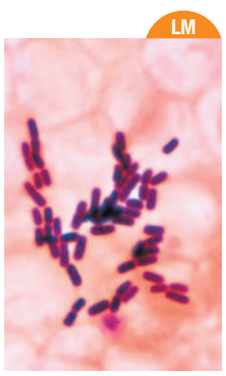
Species
(most specific)

SYMBIOTIC RELATIONSHIPS

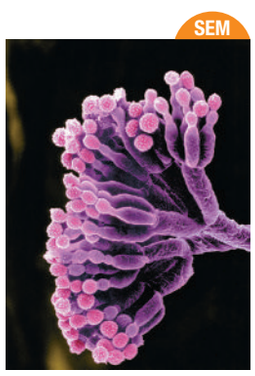


Microscopy

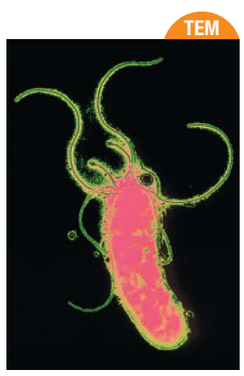
Light microscopy relies on a compound light microscope. There are many forms of light microscopy, but bright field is the most common. Electron microscopy (SEM and TEM) provides high magnification and resolution.



E. coli



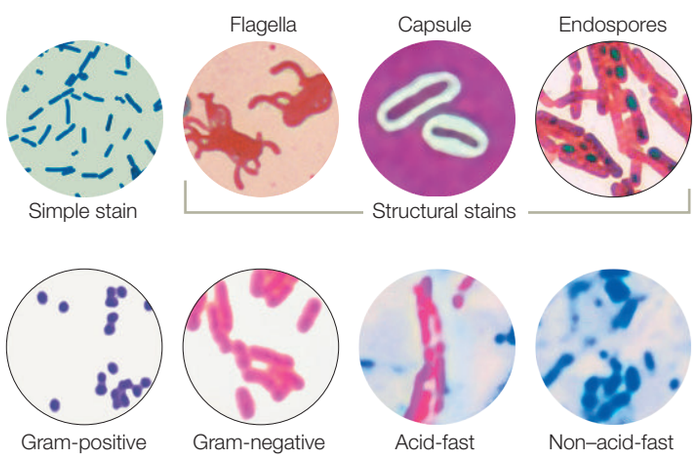
Penicillium mold



H. pylori

Staining Microbes

Simple stains give size, shape, and arrangement information. Structural stains are performed to reveal capsules, flagella, and endospores. Differential stains such as Gram and acid-fast stains distinguish bacteria based on cell wall components.



1 CHAPTER 1 OVERVIEW

1.1 A Brief History of Microbiology

- Microbiology is the study of bacteria, archaea, fungi, protists, helminths, algae, viruses, and prions. Acellular nonliving microbes include viruses and prions (infectious proteins). Cellular microbes include prokaryotes and eukaryotes.
- Endosymbiotic theory states that eukaryotic cells evolved from prokaryotic cells. Prokaryotic cells include bacteria and archaea, which are always unicellular. Eukaryotes include single-celled organisms and multicellular organisms. Animals, plants, fungi, and protists are eukaryotic.
- The golden age of microbiology encompassed important advancements, including the theory of biogenesis and the development of the germ theory of disease. Pasteur's S-necked flask experiment was central to disproving spontaneous generation. The germ theory of disease states that microbes cause infectious diseases.
- Koch's postulates of disease outline a systematic process to directly link an infectious agent to a disease.
- Aseptic practices in surgery and nursing emerged as people embraced biogenesis and the germ theory of disease.
- The scientific method is the guiding investigative principle of microbiology; it involves formulating and testing a hypothesis by making observations and drawing conclusions. Observations include any data collected. Conclusions interpret observations.
- Hypotheses and laws are united in a theory; laws are not superior to theories. Theories seek to explain how and why something occurs. A law is a concise statement or mathematical formula to predict what will occur.

1.2 Classifying Microbes and Their Interactions

- Taxonomy identifies, names, and classifies organisms using a hierarchical system.
  - The three domains are Bacteria, Archaea, and Eukarya. Just under domains are kingdoms. Species is the most specific taxonomic grouping. Strains are genetic variants of the same species.
  - The binomial nomenclature system involves naming organisms by their genus and species. Scientific names are italicized and Latinized; the first name is capitalized and reflects the genus, and the second name is lowercase and designates the species.
- Symbiotic relationships such as parasitism, mutualism, and commensalism are ecological relationships between two or more closely connected organisms.
- Our normal microbiota consists of microbes that stably reside in and on us; our normal microbiota affects our physiology—including susceptibility to opportunistic pathogens.
- Biofilms are communities of microbes that coordinate their physiological responses.
- Microbes have major roles in bioremediation, food and drug production, making important precursors for consumer products, and serving as a source for biodegradable plastics, and may even make biofuels.

1.3 Growing, Staining, and Viewing Microbes

- Growth media are mixtures of nutrients that support the growth of cells or simple organisms in an artificial setting. Media can be poured as broths, plates, slants, or deeps. Petri plates of agar-solidified media are used for isolating bacteria and generating pure cultures. Pure cultures consist of only one species.
- The streak plate technique is often used to isolate individual colonies and determine culture purity; the characteristics of the colonies on a plate can help identify a microbial species. Characterizing cultures depends on aseptic culturing techniques to limit contaminants.
- Staining specimens is central to observing and classifying microbes. Simple stains use a single dye to reveal specimen size, shape, and arrangement. Structural stains reveal flagella, capsules, and endospores. Differential stains highlight differences in bacterial cell walls in order to discriminate between distinct classes of cells.
- The Gram stain is a differential stain that highlights differences in Gram-positive versus Gram-negative bacterial cell walls.
- Gram-positive bacteria appear purple at the end of the Gram stain because their cell walls are rich in peptidoglycan and lack an outer membrane, allowing for crystal violet retention after exposure to an alcohol-acetone solution. Gram-negative bacteria have a thin peptidoglycan layer and an outer membrane that allows crystal violet to be rinsed from the cell wall when exposed to an acetone-alcohol wash. Gram-negative cells are pink after the Gram stain.
- The acid-fast stain distinguishes between cells that have mycolic acid in their cell wall (acid-fast bacteria) and those that do not (non–acid-fast bacteria). Acid-fast bacteria retain the carbol-fuchsin dye after being exposed to an acid wash and appear pink at the end of the staining procedure. Non–acid-fast bacteria lack a waxy cell wall and are stripped of the carbol-fuchsin by an acid-containing solution; these cells appear blue at the end of the staining procedure.
- The compound light microscope is the most common type of optical microscope.
- Resolution is the ability to distinguish two distinct points as separate. Immersion oil enhances resolution in light microscopy by ensuring that the light that interacts with the specimen is smoothly funneled toward the high-power objective lens instead of being scattered.
- TEM is the most common form of electron microscopy; it generates images of internal cell structures. SEM produces three-dimensional images of surface structures; it has lower magnification and resolution than TEM.
- Fluorescence microscopy requires a fluorochrome and a specialized microscope that illuminates samples with UV light.

COMPREHENSIVE CASE

The following case integrates basic principles from the chapter. Try to answer the case questions on your own. Don't forget to be S.M.A.R.T.* about your case study to help you interlink the scientific concepts you have just learned and apply your new understanding of microbiology principles to a case study.

***The five-step method is shown in detail below. Refer back to this example to help you apply a SMART approach to other critical thinking questions and cases throughout the book.**

• • •

Cholera is an acute, infectious diarrheal illness that affects millions and kills up to 120,000 people every year. It likely originated in ancient India. Starting in the 1800s, it spread worldwide through ships' bilgewater. The first occurrence in Europe and the Americas spurred a flurry of theories about the cause of the disease. The miasma, or "harmful air," theory became a popular explanation. This theory was based on the observation that air smelled bad in poverty-stricken areas where cholera was common. The miasma theory, though inaccurate, pressed public officials to address sewage issues that plagued poor areas. As sewage management improved, the stench that miasma proponents felt caused cholera decreased; the number of cases also decreased. This was interpreted as further support for the miasma theory.

In 1849, English physician John Snow observed cholera cases clustered around a local well. He ordered the pump handle removed so water could not be drawn from it, and soon after, the local outbreak ended. Despite Snow's success, the miasma theory of cholera remained popular. In 1884 Robert Koch isolated a unique, comma-shaped bacterium from stool samples of cholera patients. Koch demonstrated that this bacterium, now called *Vibrio cholerae*, was always present in cholera patients. He described how to isolate and grow it and proposed it made a toxin that caused pathology. It was later determined that *V. cholerae* is a Gram-negative, flagellated bacterium that indeed makes a potent toxin.

Despite Koch's convincing evidence, many people continued to believe the miasma theory of cholera. One especially vocal advocate was Max von Pettenkofer, a renowned chemist and public hygienist who claimed it was an oversimplification to think that *V. cholerae* alone could cause disease. He noted that Koch was unable to infect animals with the isolated bacteria and therefore failed to fulfill his own third postulate of disease. Pettenkofer argued that the bacteria were harmless until they had a maturation period in the soil and released a toxic airborne miasma that had to be inhaled in order for infection to be established. To dramatically uphold his point, in the presence of several witnesses, Pettenkofer drank a preparation of *V. cholerae* that Koch himself had isolated from the stool sample of a dead cholera patient. The 76-year-old Pettenkofer won a victory for miasma theory when he did not develop full-blown cholera. While this minor victory sustained the miasma theory a little longer, it was eventually debunked, and the theory of biogenesis was embraced.

We now recognize that *V. cholerae* is a naturally occurring waterborne bacterium that invades humans through contaminated drinking water and foods. There are hundreds of *V. cholerae* strains, but the O1 and O139 strains are responsible for most outbreaks. The O139 strain has a capsule; the O1 strain does not. *V. cholerae* is not native to the human intestinal tract and tends to inhabit the human gut only temporarily, making a long-term carrier state extremely rare. While humans can spread cholera in feces, the source of initial infection that jump-starts outbreaks comes from the natural marine environment.

Copepods are small crustaceans found worldwide in estuaries, rivers, and ponds; they naturally harbor *V. cholerae* in their gut and as biofilms on their surface. These crustaceans appear to be important in the natural ecology of the bacteria. In the presence of live copepods, the population of *V. cholerae* rapidly increased by 100-fold, while in the presence of dead copepods the population showed a modest initial increase and then rapidly declined. An unrelated bacterium, a species of *Pseudomonas*, had a comparable increase in population in the presence of dead or live copepods. Electron microscopy has shown that *V. cholerae* adheres to the surface of these tiny zooplankton creatures, especially around the oral region and egg sacs. The bacteria secrete a substance that helps the egg sac rupture to release the eggs that are ready to be fertilized, and the bacteria benefit by having an environment rich in food and also safe from protozoans that normally graze on marine bacteria. Additional studies have shown that in response to starvation, *V. cholerae* colonies growing on agar plates change from their typical smooth morphology to wrinkled (or rugose) colonies. Bacteria in rugose colonies produce a slimy substance that makes them better adapted to forming biofilms on a variety of surfaces. Despite over 100 years of studies, we still have an incomplete picture of the pathophysiology of these bacteria and how biofilms play a role in infection.

• • •

CASE-BASED QUESTIONS

1. Population studies show type O blood is practically extinct in populations from the Ganges River delta in India. Based on this chapter, provide a possible evolutionary explanation for this.
2. Provide a possible explanation for why Koch experienced difficulties in establishing an animal model for cholera.
3. What type of electron microscopy was most likely used to obtain the data about *V. cholerae*'s colonization of copepod external surfaces? Explain your reasoning.
4. We now know the miasma theory is wrong, so how did proper sewage management help reduce cholera cases, if not by reducing the stench?
5. How would you define the symbiotic relationship between copepods and *V. cholerae*? Explain your definition.
6. How would you describe the biota classification or status of *V. cholerae* in humans? How about in copepods?