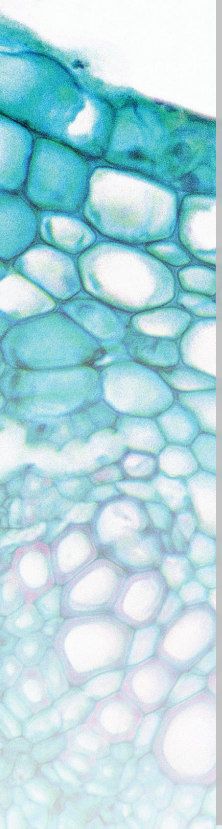

Microbiology for Surgical Technologists

Third Edition

Margaret H. Manning Rodriguez,
CST, CSFA, FAST, M.Ed.

CENGAGE

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Microbiology for Surgical Technologists,
Third Edition
Margaret H. Manning Rodriguez

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DEDICATION

I dedicate this book to my husband of 39 years, George, who has dedicated his professional life of 42 years to being the quintessential example of what a Certified Surgical Technologist should be. I also dedicate this book in loving memory of our grandson Joseph, taken much too soon. Lastly, I dedicate this to all the victims of the COVID-19 pandemic, their loved ones, and the incredible front-line responders of all types who have heroically cared for those in need.

Contents

Preface

The Importance of Microbiology for Surgical Technologists

Few healthcare professionals focus as much attention on the war against infectious disease transmission as surgical technologists. Every task, every technique, every procedure, and every movement within the surgical environment of care requires incredible awareness of potential risks and attention to the smallest details to create, protect, and maintain the sterile field—at the center of which is the surgical patient. Equally important is the necessity for surgical technologists and all members of the team to protect themselves from exposure to infectious and pathogenic microorganisms within the operating room suite and the broader outside world in which we live. Without the fundamental knowledge of microbiology and its relationship to healthcare and surgical technology, the concepts and techniques are meaningless ideas and exercises taught in a classroom or lab.

Part of a crucial foundation for perioperative care, *Microbiology for Surgical Technologists*, Third Edition helps surgical technology students understand and prevent disease transmission in clinical settings. In addition to exploring the vast microbial world, learners investigate the infectious disease process and disease pathologies, correlating them with anatomical body systems. Health and safety procedures are important topics, with key procedures for protecting patients, team members, and the students themselves. *Microbiology for Surgical Technologists*, Third Edition is also packed with helpful extras, including colorful photos, realistic case studies, end-of-chapter questions, and special boxed features that call out interesting facts and anecdotes to highlight the importance of aseptic and sterile techniques in various types of surgical intervention.

New Material in the Third Edition of *Microbiology for Surgical Technologists*

In the two decades since the first edition was released, there has been an explosion of scientific and media attention toward topics such as the following: global epidemics of viral diseases jumping from other species to humans; expanding microbial antibiotic resistance; federal insurance regulations regarding healthcare-associated infections; societal debates regarding immunizations and impact on public health; development of bioterrorism agents as weapons of mass destruction; resurgence of previously eradicated diseases; identification of microbial species mutations; and emergence of previously unknown diseases with dramatic impact and mortality rates.

The third edition of *Microbiology for Surgical Technologists* expands on on these topics with:

- Updated content including historical timelines, current world events (including information on COVID-19 in multiple chapters), challenges for healthcare providers, and impact of disease transmission on individuals and society at both the local and global levels.
- Alignment of material with the AST Core Curriculum for Surgical Technology, Seventh Edition.
- Revised chapter Learning Objectives with real-world relevance and broader professional contexts.
- New "Under the Microscope" scenarios and review questions for learning assessment.
- New and diverse images and graphics for enhancement of subject matter materials.

Chapter Overview

Chapters have been developed with emphasis on examination of general and consolidated microbial classifications as well as the correlation between indigenous microflora and body systems with a wide selection of color photos, graphics, and tables to illustrate subject materials. A progression of information includes the following: introduction to the science of microbiology and the laboratory; classifications of microbes into eukaryotes, prokaryotes, viruses, parasites, Gram-positive cocci and bacilli, Actinobacteria, Gram-negative cocci, spirochetes, and bacilli; microbial growth and viability; genetics and mutations; disease transmission; control of microbial growth; microbiologically-linked pathology of specific body systems; and emerging, reappearing, and recurring diseases.

Key Features for Students and Instructors

Careful attention has been given to the correlation of all content with the Core Curriculum for Surgical Technology, Seventh Edition so instructors are assured of compliance with current education and accreditation requirements and students are prepared for certification examination content questions.

Textbook Features:

- Big Picture questions guide students' focus and attention toward general subject areas of discussion.
- Clinical Significance Topics (CSTs) link the chapter material to specific surgical technology skills, techniques, and responsibilities.
- Under the Microscope case studies at the end of chapters use relevant scenario questions to assess comprehension and critical thinking about the material and its connection to the perioperative environment.
- Micro Notes provide quirky and novel tidbits of information about the microbial world and our relationship with it.

MindTap Features:

- Microbes in the Media get students engaged with a short news video showing how microbiology is relevant in the world today.
- Concept Checks in the ebook assess students' understanding of key concepts as they read.
- Flashcards help students learn key terms.
- PowerPoint Reviews summarize key concepts from the chapter.
- Video Quizzes show how chapter concepts apply in the real-world and assess students' understanding.
- New Branching Activities present real-world scenarios in which students choose an action and react to the consequences.
- End of Chapter Quizzes assess students' understanding of key concepts.
- Certification Exam Review provides a practice assessment to help prepare students for the certification exam.

Student Outcomes

Surgical technology students will gain an understanding of the methods of identifying, classifying, and testing for various groups of microbes that determine the appropriate course of treatment for the pathological conditions created by the various infectious agents. Topics such as personal protective equipment (PPE) use, hand hygiene, surgical conscience, care and handling of culture specimens, prevention of surgical site infection (SSI), and healthcare-associated infection (HAI) are discussed. Important correlations between the types of pathogenic microbes commonly encountered in surgery and the potentially life-threatening results are covered. This knowledge will enhance performance as allied healthcare professionals and provide real-world concepts for infection prevention strategies in day-to-day life in the operating room and the local or global community. Additionally, for those interested in future medical/surgical humanitarian relief work in foreign countries, the covered topics of diseases and prevention methods available are important considerations for health maintenance.

Teaching and Learning Package

Additional instructor resources for this product are available online. Instructor assets include an Instructor's Manual, Educator's Guide, PowerPoint® slides, a test bank powered by Cognero®, and more. Sign up or sign in at www.cengage.com to search for and access this product and its online resources.

- Instructor Manual—provides chapter outlines with instruction and activity ideas.
- PowerPoint slides—support lectures with definitions, key concepts, and examples.
- Guide to Teaching Online—offers tips for teaching online and incorporating MindTap activities into your course.
- Educator's Guide—offers suggested content from MindTap by chapter to help you personalize your course.
- Cengage Testing, powered by Cognero®—a flexible, online system that allows you to access, customize, and deliver a test bank from your chosen text to your students through your LMS or another channel outside of MindTap.
- Transition Guide—outlines changes between the Second and Third editions of the textbook.

About the Author

Margaret Rodriguez has been a Certified Surgical Technologist (CST) since 1980 and a Certified Surgical First Assistant (CSFA) since 1992. She graduated from the El Paso Community College Surgical Technology program in 1980 and immediately began private-scrubbing for a neurosurgeon who took her under his wing from a busy private practice to the world of medical school academia. Following his retirement, she returned to general surgical practice. After obtaining her Associate of Applied Science (AAS) degree in Surgical Technology, she began teaching in the EPCC surgical technology program with her mentor, Cynthia A. Rivera RN, BS. Changing course from nursing studies, she earned her Bachelor of Science (BS) in Occupational Career Training and Development from Texas A&M University, Corpus Christi in 2002 and subsequently received her Master's of Education in Higher Education Leadership from the University of Texas—El Paso in 2018. She is a tenured Professor and Program Coordinator at El Paso Community College.

Professionally, Mrs. Rodriguez served on the CSFA Exam Review Committee of the National Board of Surgical Technology and Surgical Assisting (NBSTSA) and is now serving as a member of the Board of Directors. She has been a site-visitor for the Accreditation Review Council on Education in Surgical Technology and Surgical Assisting (ARC-STSA). She served on the Texas State Assembly of AST Board before being elected to national office in the Association of Surgical Technologists (AST) in 2005. During her eight years on the national AST Board of Directors, she served as Director, Vice-president, and as AST President from 2011 to 2013 and earned the title of Fellow of the Association of Surgical Technologists (FAST). During that time, she also served as Chair of the Council on Surgical and Perioperative Safety (CSPS) from 2012 to 2013 and as the AST Commissioner to the Commission on Accreditation of Allied Health Education Programs (CAAHEP) in 2012 to 2013. She is the first and currently the only CST or CSFA faculty consultant for Ethicon, a division of Johnson & Johnson Medical Devices.

She has been a contributor to the AST Exam Review Study Guide, three editions of Surgical Technology for the Surgical Technologist, Surgical Instrumentation, Alexander's Surgical Procedures, an upcoming Surgical Assisting textbook, as well as writing numerous AST journal articles and contributing to publications by AORN, NBSTSA, and Outpatient Surgery magazine.

Mrs. Rodriguez has been married for 39 years to George Rodriguez, also a CST, and together they have four children and twelve grandchildren.

Acknowledgments

I thank my husband, family, and friends for their unwavering encouragement and understanding of the considerable time needed to complete this project.

I thank my mentor Cynthia A. Rivera, RN, BS, for giving me the foundation for my professional surgical technology career and owe my first employer and dear friend, the late William J. Nelson, MD, enormous gratitude for giving me the freedom to expand my skills and develop my passion for the world of surgery and healthcare education.

I appreciate every student I've had the honor to instruct over the past 26 years for reminding me to step back and look at things from the perspective of someone new to the mysteries and complexities of the perioperative environment.

I sincerely thank the members and staff of AST for their dedication to quality surgical patient care and giving me the opportunity to serve our professional organization and Ethicon for recognizing surgical technologists and first assistants as valuable and irreplaceable surgical team members and partners with industry for deliverance of quality patient care.

Finally, I am forever grateful to all of the wonderful members of the Cengage Learning team who guided me through the complex world of publishing with patience and understanding.

Reviewers

The author thanks the following individuals for their careful reviews and recommendations for improvements in the manuscript. Their comments were most helpful in making this text market-ready.

Kathy Patnaude, CST, BA, FAST
Midlands Technical College
West Columbia, SC

Lisa Day, CST, CSFA, FAST
Lord Fairfax Community College
Warrentown, VA

Mark Wilms, CST, CRCST, CHL, M. Ed.
Pima Medical Institute
Denver, CO

Michael Sells
Kirkwood Community College
Cedar Rapids, IA

Robert Blackston, M. ED., CST, CSFA
North Idaho College
Coeur d'Alene, ID

Sugey F. Briones, CST, BHA
American Career College
Los Angeles, CA

CHAPTER 1

Introduction to Microbiology

Learning Objectives

After completing the study of this chapter, you will be able to:

1. Define key terms.
2. Discuss the responsibilities of surgical technologists and other sterile surgical team members in prevention of disease transmission.
3. Discuss significant historical contributions from pioneers in microbiology.
4. Relate notable discoveries and events of the twentieth century from the historic timeline to current, twenty-first century public health concerns.
5. Explain how the theories of spontaneous generation and abiogenesis were disproved and the impact on the work of future researchers.
6. Discuss the scientific impact of Koch's postulates, including the exceptions to them.
7. Apply critical thinking skills in relating chapter material to the surgical environment of care or broader global community.

Key Terms

Abiogenesis
Aerobic
Anaerobic
Antibiotic
Aseptic technique
Bioterrorism
Blood-borne pathogens (BBPs)
Cell theory
Chemotherapy
Conjugation
Endemic
Epidemic
Etiology
Germ Theory of Disease
Germ warfare
Gram stain
Immunity
Immunocompromised
Inoculation
Koch's postulates
Other potentially infectious materials (OPIMs)
Pandemic
Pasteurization
Penicillin
Personal protective equipment (PPE)
Petri dish
Puerperal fever
Pure culture technique
Quarantine
Sterilant
Surgical conscience
Vaccination

The Big Picture

History and microbiology may not be your favorite subjects, especially learning about a bunch of long-dead scientists who made discoveries that we now take for granted. The COVID-19 pandemic upended global life starting in 2020 and showed us how events of the past can repeat in the present with both remarkably similar and quite different impact and responses. Keeping an open mind may actually help you find the information interesting and broaden your understanding of the wonders and interconnectedness of the world around us from microscopic to macroscopic points of view.

During your examination of the topics in this chapter, consider the following questions:

1. Which pioneers of microbiology had the biggest impact on surgical patient care?
2. What types of discoveries in this century, in your opinion, equal those made by the pioneers?
3. Do patterns exist for disease outbreaks, treatment, eradication, and recurrence and in what ways are those patterns beneficial or detrimental to global health responses?
4. How does awareness and understanding of the mechanisms of disease transmission help to break the chain of infection?
5. Based on your study of the topics covered, do you feel that public health and prevention of disease transmission will become a larger focus in daily life and what would that focus look like?

Microbiology and Surgical Technology

The most fundamental component of surgical technology is providing the best possible care for the patients who come into our operating room suites. Many surgical procedures are increasingly complex and technical, but no matter what type of procedure or variety of instruments and equipment used, the delineation between what is sterile and what is unsterile is of paramount importance and may determine the ultimate outcomes for these patients. The concept of a **surgical conscience**, to which surgical technologists hold so tightly, is largely based on a broad educational foundation and understanding of microbiology and its relation to disease transmission from recognized or undetected sources of contamination. This guiding principle ensures that patients entering the surgical environment will receive optimal care with the goal of positive postoperative outcomes. Microbial contamination of a surgical wound, cross-contamination between patients or the environment, and the emergence or re-emergence of diseases among the general population may be minimized through a thorough understanding of:

- Classes of microorganisms and their ability to cause disease
- Various mechanisms for the spread of pathogenic microorganisms
- Aseptic and sterile techniques designed to prevent contamination
- Appropriate diagnosis and pharmacological treatment of disease
- Personal and community responsibility for utilization of resources to prevent **endemic**, **epidemic**, or **pandemic** outbreaks of disease

A minor bacterial or viral infection may be merely an annoyance to a healthy individual; however, to **immunocompromised** patients such as those who routinely enter the surgical environment of care, it may become literally a matter of life and death. Surgical technologists must also be responsible for their own health and safety.

Clinical Significance Topic

Surgical technology is a profession heavily dependent on scientific research. It is the cumulative knowledge of all the scientists who have studied microorganisms and diseases that gives validity to our professional practice and dedication to the principles of asepsis. Without knowledge of the incredible efforts of these pioneers, we might not understand the reasons we are taught to pay such close attention to the importance of sterile technique. It is not possible to simply see whether an item is sterile or unsterile, but we can have reasonable assurance that if our technique is stringent and we use our surgical conscience, then the patients in our care will have the best outcomes possible. *Aeger Primo*—the patient first!

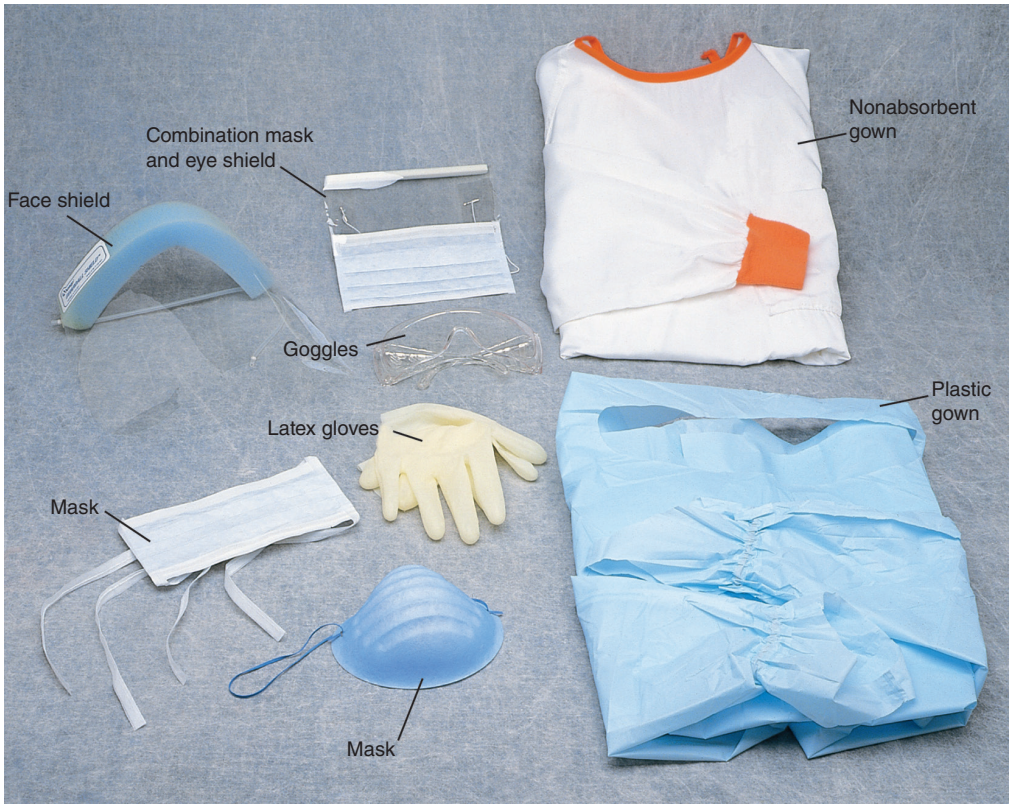


Figure 1-1 Examples of personal protective equipment (PPE).

Personal protective equipment (PPE) and knowledge of practices that reduce or prevent exposure to **blood-borne pathogens (BBPs)** or **other potentially infectious materials (OPIMs)** are important tools in the surgical technologist's professional armamentarium (see Figure 1-1).

The news media covers the outside world with a much broader focus and stories of topics such as:

- The global COVID-19 pandemic and its morbidity and mortality statistics
- Debates over the development and efficacy of vaccinations
- The impact of politicization of public health initiatives
- Mechanisms for disease transmission and preventative measures
- Food recalls after consumers are sickened
- Fears of use of biological weapons of mass destruction
- Exposure of patients to contaminated drugs or surgical instruments
- Cases of diseases turning up in travelers to foreign countries and then returning to the United States, potentially exposing fellow passengers over long distances in close spaces
- Re-emergence of childhood diseases previously thought eradicated due to lack of parental compliance in immunization schedules
- Cases of diseases spreading from animal populations to humans

Whether in the smaller, controlled operating room environment or on a more global scale, surgical technologists are uniquely qualified and skilled to fight the battle against microscopic armies of invaders. Breaking the chains of infection requires surgical technologists to practice standard techniques and utilize the comprehensive education gained through the core curriculum (including the study of microbiology) each day and on every procedure.

Early Pioneers of Microbiology

Taking time to look back in history to examine the origins of a science, especially from a twenty-first century perspective, demonstrates that the enormity of the accomplishments of the trailblazers in microbiology. This section examines the most prominent examples of these early scientists and their determination to give proof to theory by replacing mysticism and folklore with experimentation and scientific methodology.

Girolamo Fracastoro

There have been a number of individuals over the centuries who have studied and tried to demonstrate the existence of living organisms responsible for the spread of disease. One of the earliest pioneers was a physician from Verona, Italy, named Girolamo Fracastoro.

Fracastoro published his research regarding syphilis in 1530. In 1546, he published his findings regarding epidemic

diseases in a paper entitled, *De Contagione et Contagiosis Morbis* (*On Contagion and Contagious Diseases*). He theorized that tiny, unseen organisms were spread by several means including contact between an infected host and others, indirect contact, carried on clothing, or carried through the air. Fracastoro was the first to apply scientific principles to his theory. Although his findings were generally accepted at that time, following his death in 1553, they soon fell out of favor and scientific focus until the late 1800s.

Robert Hooke

A pivotal event occurred in the mid-seventeenth century when a prolific English scientist, Robert Hooke, designed and built a compound microscope. He built telescopes to study the heavens and later used his talents to focus on much smaller bodies. In 1665, he published a work entitled "Micrographia" (small drawings) in which he was the first to use the term "cell" to describe the small, honeycomb-like spaces found in cork. Hooke's discovery was the beginning of **cell theory**—that all living things are composed of cells. Hooke was better known for his subsequent research in which he postulated that the ability of something to be deformed by application of stress forces and return to its original size, shape, or form when those forces are removed is the property of elasticity. Hooke's Law in its mathematical equation was published in 1676 and is still in use today.

Antonie van Leeuwenhoek

Dutch amateur scientist Antonie van Leeuwenhoek was the first to observe and record bacteria and protozoa in 1673. He used a single-lens microscope and a variety of sources such as rainwater, saliva, and even his own semen, to examine what he termed "animalcules" (see Figure 1-2). He reported his results to the Royal Society of London between 1673 and 1723, including the first accurate drawings of various types of bacteria, using only a simple microscope (see Figure 1-3). These drawings served as the basis for modern depictions of the previously "invisible" world.

Francesco Redi

Francesco Redi, an Italian physician and biologist set out to disprove the theory of **abiogenesis** in 1668, even before Leeuwenhoek's findings were reported. Redi, openly critical of the theory, devised an ingenious experiment in which he filled three jars with decaying meat and sealed them with a lid. He also placed meat in three other jars but left those open. Maggots soon appeared on the meat in the open jar, but no maggots were present in the sealed jars. Redi concluded that given ready access to the meat, the flies had laid their eggs there and could not do so on the meat in the other jars because they were sealed. His opponents were undaunted and scoffed at the results of the experiment, arguing that fresh oxygen was a requirement of spontaneous generation.

Redi then conducted a second experiment. Again, meat was placed in three open jars, but this time the other three jars were sealed with fine mesh gauze to satisfy his critics. The results were the same as the first experiment. This provided a strong basis for refuting abiogenesis, but the scientific community was still not ready to give up its long-held belief.

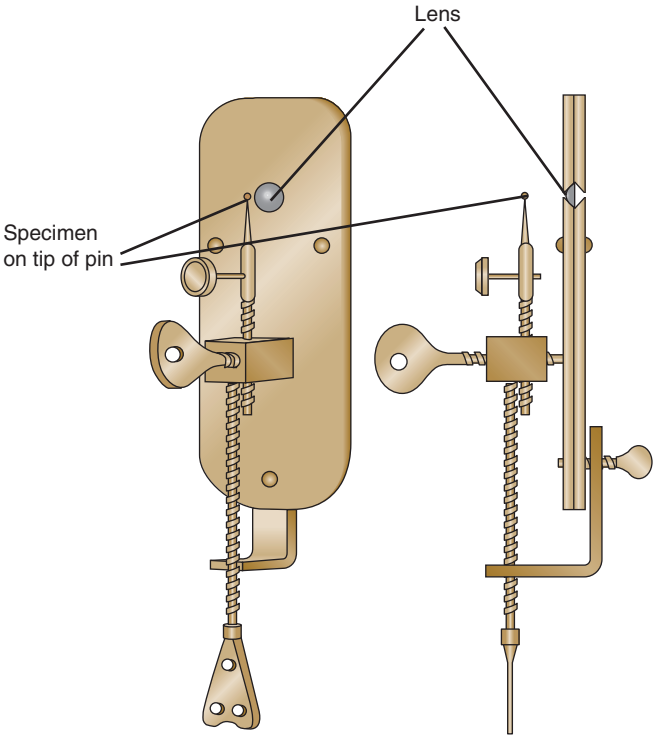


Figure 1-2 Antonie van Leeuwenhoek's microscope. The specimen was placed on top of the point in front of the small lens.

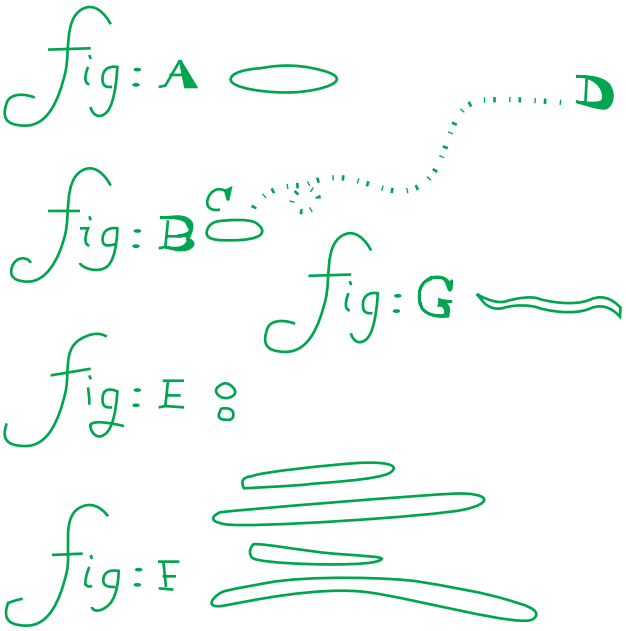


Figure 1-3 Van Leeuwenhoek's drawings of cells that were later identified as bacteria.

John Needham and Lazzaro Spallanzani

Most scientists believed in the theory of spontaneous generation, or abiogenesis, until the second half of the nineteenth century. This theory claimed that life could spontaneously arise from non-living material. An English priest, John Needham, had performed experiments in 1749 with meat broths that became infested with microbes, spurring his belief in a "vital force" that was responsible for the spontaneous generation of life. Other scientists were interested in finding the origins of these seemingly spontaneously generated cells after the discoveries of Hooke and Leeuwenhoek. Italian scientist and priest, Lazzaro Spallanzani, one of these skeptical researchers, sought to disprove abiogenesis through his own experiments years later showing that boiling was able to kill or prevent microbial growth but failed to sway Needham or the rest of the scientific community.

Edward Jenner

Smallpox had become a major cause of death in many parts of the developing world by 1798. Early attempts at vaccinating healthy individuals with small amounts of fluid from the pustules of infected hosts proved ineffective. The practice that originated in India and China known as variolation did not induce mild cases and immunity as hoped but instead, often caused serious infections that spread to others by contact exposure.

An Englishman, Edward Jenner became a pupil of John Hunter, a renowned physician and surgeon at St. George's Hospital in London. Hunter instilled in Jenner a scientific curiosity telling him, "Why think (speculate)—why not try the experiment?"

Jenner witnessed first-hand the devastation of smallpox throughout all areas of the country and segments of the population. He noted that individuals who became infected with a much less serious disease, known as cowpox, a disease transmitted from exposure to cattle, would easily recover and those individuals never contracted smallpox, even after intentional exposure. Jenner put his critical thinking and experimentation talents to use when he **inoculated** an 8-year-old boy with fluid from the blisters of a milkmaid with cowpox. The boy became mildly ill and recovered. Jenner then inoculated the same boy with smallpox; however, he never displayed any signs of infection. Jenner was eventually recognized prior to his death in 1823 as having been the first to effectively provide **immunity** through **vaccination**, despite problems including others trying to take credit for Jenner's work as well as difficulty in creating and then transporting properly prepared cowpox vaccine doses to the rest of Europe and America.

Ignaz Semmelweis

Another mid-nineteenth century pioneer was Ignaz Semmelweis, a Hungarian physician, who worked in an obstetrics clinic in Austria and witnessed a 25–30 percent maternal fatality rate from **puerperal fever**. After he experienced the death of a friend from a wound infection, he focused his observations on the practices of staff members and students in the clinic. He discovered that the patients attended to by midwives had much lower infection rates than those who were seen by medical students who would often participate in autopsies and anatomical dissections prior to examining the patients on the obstetrics ward. His investigation showed that the midwives took great care to wash their hands often and between patient examinations, whereas the medical students took no such steps.

Once determining the root cause, Semmelweis implemented practices of routine hand washing with chlorinated lime solutions. The result was a dramatic reduction of maternal mortality from more than 18 percent down to just over 1 percent. Unfortunately, the dedication to routine hand washing was not embraced by other physicians. Following years of working at other obstetrical hospitals in other countries, the physician population remained resistant to Semmelweis' findings and recommendations. He eventually was institutionalized after a mental breakdown and ironically, died in 1865 from a surgical wound infection following a minor procedure in 1865.

The Golden Age of Microbiology

The 60 years between 1855 and 1915, saw major strides in the study of microbiology in an increasingly enlightened era that embraced experimentation and the scientific method of proving new, or disproving old and commonly accepted, beliefs about the origins and spread of diseases.

Louis Pasteur

One of the most recognized figures of the Golden Age of Microbiology is Louis Pasteur, a French scientist, who earned his doctorate in physical sciences with a focus on chemistry and physics. As an educated scientist, Pasteur felt compelled to dispute the theory of abiogenesis, which had been widely accepted up until the latter part of the 1800s (see Figure 1-4).

Everett Historical/Shutterstock.com.

Figure 1-4 Louis Pasteur, 1822–1895.

Pasteur conducted a controlled experiment by using short-necked flasks. He filled several of the flasks with beef broth and boiled the broth. Some of the flasks were left open and, consequently, microbes were found thriving in the broth. As expected, the sealed flasks remained free of microbes.

Next, using flasks with necks bent into the shape of an "S," Pasteur again boiled beef broth and allowed it to cool in the flasks without sealing them (see Figure 1-5). Microbes never appeared in the S-shaped flasks, allowing Pasteur to conclude that the curve in the neck of the flasks had trapped

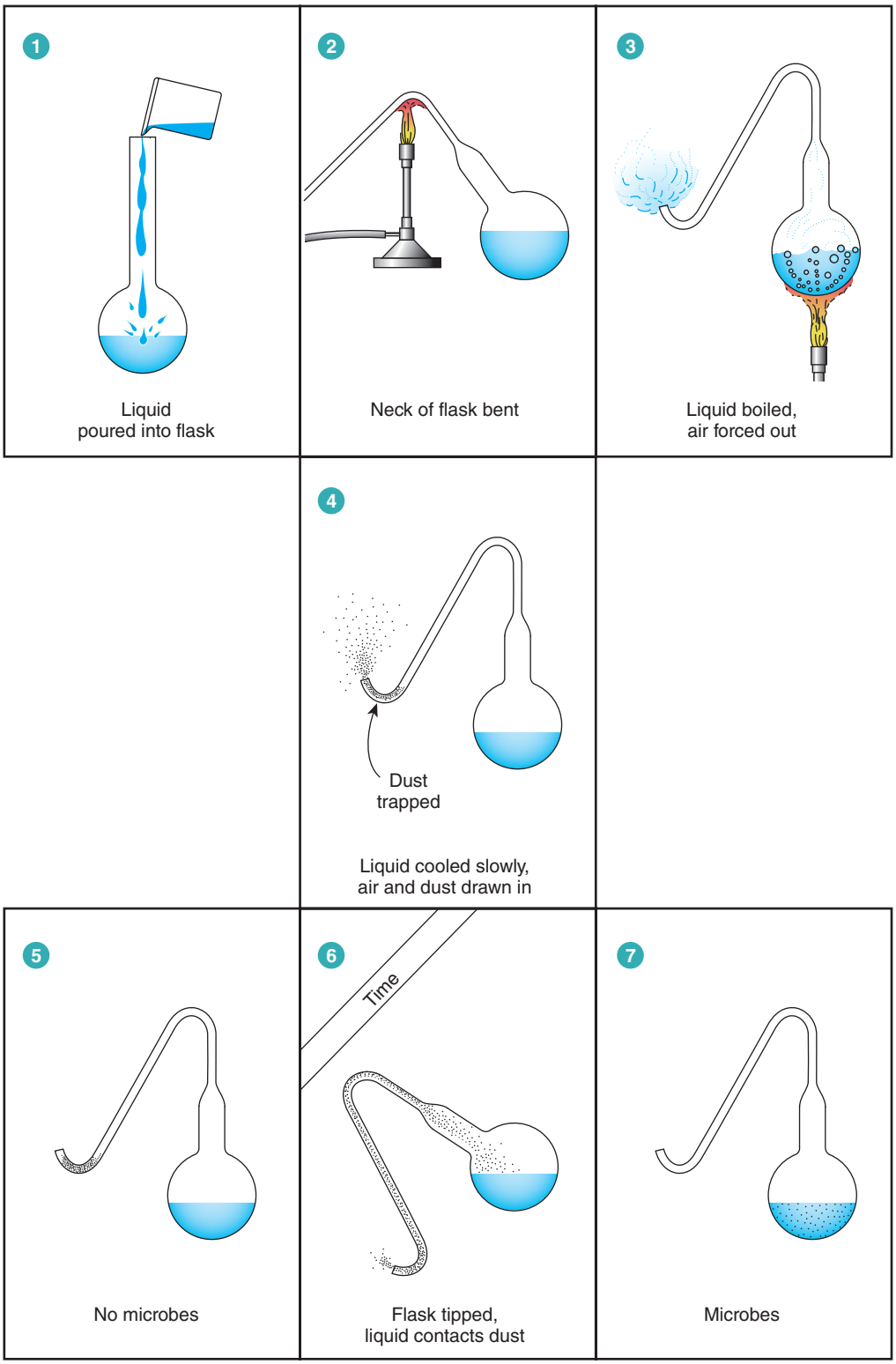
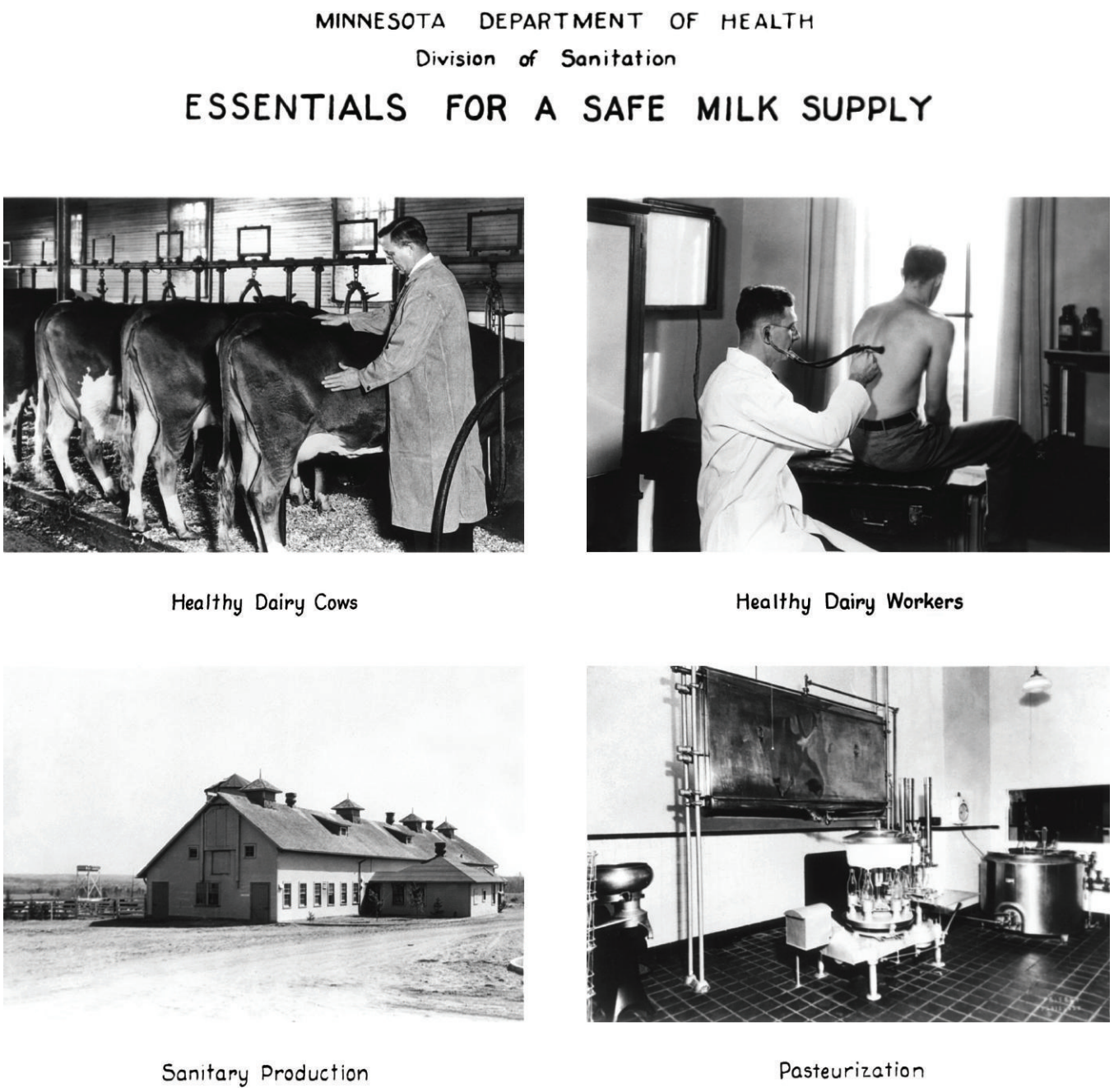


Figure 1-5 Pasteur's experiment disproving the theory of spontaneous generation.

the airborne microbes, preventing the contamination of the broth. Pasteur had successfully refuted Needham's "vital force" theory, permanently dismantling the theory of spontaneous generation.

Louis Pasteur is probably most notably credited with developing experiments leading to the Germ Theory of Fermentation. French Emperor Napoleon III commissioned a study of distilling processes. Pasteur's experiments showed that bacteria were the agents responsible for the spoilage of beer and wine. He was able to demonstrate that the bacteria changed the alcohol into acetic acid, otherwise known as vinegar. He identified for the first time that microorganisms can be categorized into either **aerobic** or **anaerobic** classifications after unexpectedly arresting the fermentation process by passing air through the liquids, demonstrating that certain types of microbes cannot survive in the presence of air.

His solution to the problem was to use just enough heat to kill the bacteria without affecting the taste of the product. The same heating process is used today to kill bacteria in milk and is referred to as **pasteurization** (see Figure 1-6). Pasteur's proof of the relationship between food spoilage and microorganisms was a major contribution to the establishment of the connection between disease and microbes.



Courtesy of CDC/ Minnesota Department of Health, R.N. Barr Library, Librarians Melissa Rethlefsen and Marie Jones.

Figure 1-6 1934 instructional flyer for maintenance of a safe milk supply, from the Minnesota Health Department.

Before Pasteur, treatments for some diseases were established without knowledge of their **etiology**. Pasteur's discovery of the link between yeasts and alcohol established an entirely new point of view within the scientific community. Scientists began pondering the possibility that microbes might actually cause illness, launching the idea known as the **Germ Theory of Disease**.

For many, this radical theory that suggested unseen microbes were responsible for disease was simply inconceivable. Scientists of this Golden Age accumulated a wealth of information to support the theory. For example, in 1865 Pasteur discovered that a protozoan was responsible for a devastating silkworm disease. He then developed the technique for recognizing the silkworm moths that were affected by the protozoan. Pasteur was faced with a professional dilemma in 1885, after several years studying rabies, a rhabdovirus carried in the saliva of infected host animals. The parents of a young boy bitten by a rabid animal knew of Pasteur's research involving developing vaccines against rabies and implored him to treat their son. Pasteur, who was not a medical physician, took a professional and legal risk in administering his experimental rabies vaccine to the boy who survived and never developed the rabies infection.

Joseph Lister

Joseph Lister, the English surgery pioneer, was responsible in the 1860s for establishing some of the first principles and practices known as **aseptic technique** to be used in the operating room. Lister recognized the significance of Pasteur's findings, most importantly the link between microbes and animal diseases. Lister knew that carbolic acid (phenol) killed microbes, so he began directly treating surgical wounds with a phenol solution and placing phenol-soaked dressings on the wounds. His infection rate dramatically decreased.

Lister also used phenol as an antiseptic, cleansing the skin of the patient prior to surgery. His surgical team used phenol as a hand scrub prior to surgical procedures. The solution was also used as a **sterilant** aerosol that was sprayed over the surgical field to prevent patient infections.

Lister's contributions to the aseptic technique did not end with the use of phenol. He was a proponent of wearing gloves during surgery, changing gowns or aprons between cases, and cleaning and disinfecting surgical instruments by boiling before they were to be used on the next patient. These routines are all basic but critical measures the modern surgical teams could not conceive of omitting.

Robert Koch

The German physician, Robert Koch was one of the most influential bacteriologists in the history of medicine and epidemiology. He studied under Friedrich Gustav Jacob Henle, a German anatomist, whose research in the 1840s included attempts to disprove the miasma theory and humoral doctrine of disease established in the times of the ancient Greeks.

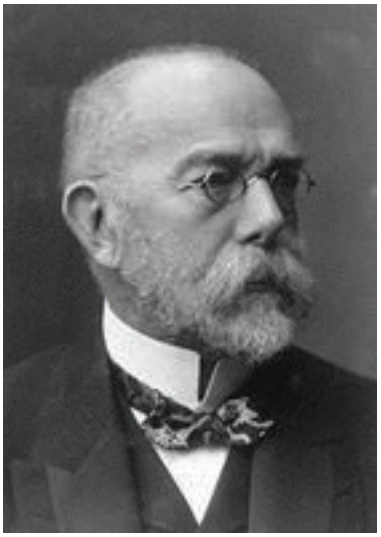
Henle believed that disease was caused by microorganisms, and this may have been the foundation for his protégé's future work. In 1876, Robert Koch became famous in the medical community for his research showing a causal relationship of rod-shaped bacteria, now known as *Bacillus anthracis*, with the blood of cattle that had died of anthrax. He cultured the bacteria and injected samples of the culture into healthy animals. When the animals became sick and died, he took samples of their blood, isolated the bacteria, and compared it to the original bacterial samples. He found that the two blood cultures contained the same bacteria. Koch thus established a sequence of steps for experimentally proving that a specific microbe causes a specific disease (see Figure 1-7).

These steps, called **Koch's postulates**, sometimes referred to as the Henle-Koch Postulates, were first discussed in a publication in 1877 and are as follows:

1. The same disease-causing microorganism must be observed in all cases.
2. The pathogen must be isolated and grown in pure culture.
3. The pathogen from the pure culture must reproduce the disease when inoculated into a susceptible animal.
4. The microorganism must be isolated from the inoculated animal and proven to be the original disease-causing pathogen.

There are exceptions to Koch's postulates including:

1. Many types of microbes, such as viruses and *Rickettsia* cannot be grown *in vitro* in the laboratory on artificial media. Therefore, the pathogens are grown in cultures of various types of living human or animal cells, within chicken eggs, or in different types of non-human or non-animal tissues.



Courtesy of the National Library of Medicine.

Figure 1-7 Robert Koch, 1843–1910.

2. As stated in the third postulate, to induce the disease from a pure culture, the animal must be susceptible to the pathogen. Some animals, such as rats, are resistant to many microbial infections. Other pathogens are species-specific, meaning they thrive in only one type of animal. For ethical reasons, it is not acceptable to inject humans with a known pathogen, and the researcher may only be able to observe the pathogen in human cells that can be grown in the laboratory.
3. Some diseases or infections occur only when an opportunistic pathogen can infect the host. An example is a pneumonia infection secondary to influenza. Scientists specifically searching for the influenza virus, might be misdirected by instead isolating the pneumonia-causing bacteria.

Koch's postulates are still used as an important basis for research in the laboratory. The investigation of pathogens that have the capability to cause several diseases, however, requires a combination of clinical signs and symptoms and laboratory methods to isolate the pathogen and the specific disease process it causes.

The world of science and medicine, as with many prestigious professions, spawned fierce competition, rivalry, and even political conflict. In the early 1880s, Koch and Pasteur were both famous in their own countries and the scientific community but were locked in a bitter battle for recognition for the discovery of the relationship between disease-causing bacterium *B. anthracis* and its disease manifestation, anthrax. Pasteur was able to achieve a vaccine for anthrax in 1881.

In the same year, Koch was able to produce a solid agar made of an extract of red marine algae, as a growth medium for bacterial cultures. The solid agar was prepared and placed into small plates designed by his laboratory assistant, Julius Petri. These small plates referred to as **Petri dishes** are used even to this day for culture tests. This method replaced the previous liquid broths used by the early scientists.

Koch also developed the **pure culture technique** that is still in use in today's modern laboratory. Koch placed sterilized slices of potato into a sterilized glass container, using the lid as a medium to culture bacteria. But he discovered that not many types of bacteria grow on potatoes.

Koch then concocted a meat broth and gelatin medium, coating it with a bacteria-containing material. After incubation, Koch isolated the bacterial colonies that are visible without the use of a microscope and transferred a portion of that colony to a fresh medium using a wire loop that had been heat-sterilized by a flame. He then incubated the new colony.

Koch developed the streak plate method, in which a wire loop is used to "streak" a pattern over the nutrient medium (see Figure 1-8). As the pattern is traced, bacteria are rubbed off the loop onto the medium in fewer and fewer numbers. The last few microbes are far enough apart to grow into isolated colonies. Koch's demonstration proved that one kind of bacterium in pure culture could be obtained from a sample containing a mixture of microbes.

The value of Koch's development of the pure culture technique cannot be understated. If it were not for this contribution to microbiology, the research of bacteria could not have advanced to its present form.

The establishment of the pure culture technique also contributed to the field of bacterial classification. Koch established that separate species of bacteria do exist and that the categories are not interchangeable. Before Koch's research, it was thought that all species of bacteria were the same and merely changed due to differing environments. The pure culture technique ended that theory.

A few years following the acceptance of the superiority of the solid agar culture medium, a Danish physician, Hans Gram, developed a bacterial staining method in 1884. The **Gram stain**, as with the Petri dishes with agar and wire-loop streak technique, continue to be routine methods used by laboratories in the twenty-first century. The Gram stain procedure allows for differentiation between bacteria through identification of qualities of their cell wall thickness, composition, and the degree of retention of the stain. Bacteria are classified through this process as being either Gram-positive or Gram-negative. Staining techniques are discussed further in Chapter 3.

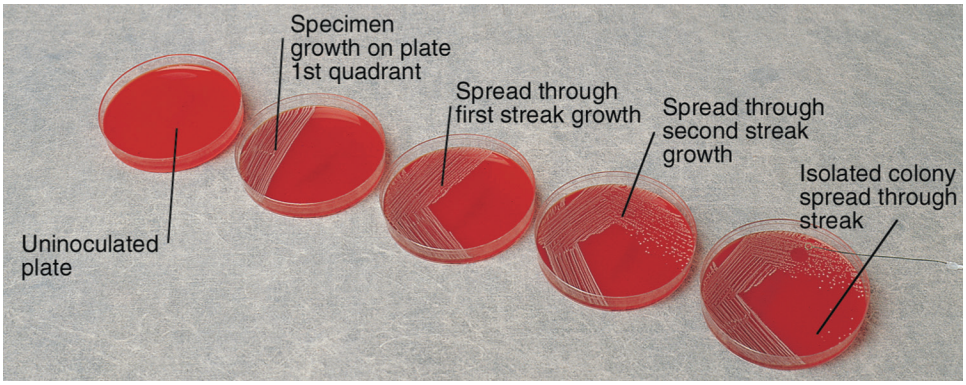


Figure 1-8 Streaking and inoculating patterns on blood agar.

Koch felt the pressure to remain relevant among his peers following his success with his refined laboratory techniques. In 1890, Koch culminated his extensive research on tuberculosis with news of a treatment that was seized by the media as a"cure." A small percentage of patients with mild skin symptoms caused by the tubercle bacilli gained some therapeutic benefit from Koch's remedy that he called tuberculin. Most patients saw little to no benefit and some actually suffered fatal allergic reactions. Koch's reputation suffered further when it was discovered that he had a significant financial interest in the production of tuberculin.

Despite the trouble with his research on tuberculosis, Koch continued his studies and subsequently began to focus his attention on the deadly cholera infection. Controversy plagued Koch again in the form of public challenge by Emanuel Klein, a British microbiologist, on Koch's findings and recommendations regarding quarantine of cholera victims. The scientific community ultimately agreed to acknowledge value in the work of both men and, in turn, they were able to accept components of each other's recommendations. Koch was eventually awarded the Nobel Prize for Physiology or Medicine in 1905.

Just as Koch was influenced by his teachers, several of his students went on to achieve recognition for their work as well. Emil von Behring won the Nobel Prize for Physiology or Medicine in 1901 for his work with serum therapy and application against tetanus and diphtheria, 4 years before his mentor was awarded the prize. August von Wassermann became famous in 1906 for his work with Albert Neisser to create a universal blood-serum test to detect syphilis.

Paul Ehrlich, another student of Robert Koch, would go on to become a Nobel Prize winner in 1908. He was given the name of "father of **chemotherapy**" for his work with chemical agents for the treatment of pathological microbes which were previously isolated and categorized by his scientific peers and predecessors. Ehrlich worked with derivative forms of the poison arsenic to develop what he called the first"magic bullet," a chemical therapy capable of destroying a pathogen without harming the host. Salvarsan was version 606 of the numerous arsenical compounds developed and was initially tested on rabbits infected with syphilis. Ehrlich later developed Neosalvarsan, version 914 of the drug, as a more soluble and easily administered alternative, although its curative effects on syphilis were less than the more potent original.

The Modern Era of Microbiology

The foundations for modern science had been firmly established by the time Koch and his students and colleagues had achieved public recognition for their extensive research and findings.

Ruth Ella Moore

In 1933, African-American bacteriologist, Dr. Ruth Ellen Moore was the first African-American to receive a Ph.D. in bacteriology. She also was the first African-American to join the American Society for Microbiology in 1936. Her doctoral research focused on treatments for tuberculosis, which at the time was the second highest cause of death in the United States. She later expanded her work to immunology and blood type-associated pathology in African-Americans. She became the head of the Howard University's Department of Bacteriology.

Moore's dissertation research contributed to eventually treating tuberculosis, which was the second leading cause of death in the U.S. at that time. She has also published work on immunology, dental caries, and blood types in African-Americans. She lectured in bacteriology at variety of universities, including teaching at and being the head of the Department of Bacteriology at Howard University. Eventually in 1986, she was recognized by the ASM Minority Committee for her exemplary service as a mentor, leader, and activist in the microbiology science community.

Elizabeth Bugie

Elizabeth Bugie, an American microbiologist and biochemist was part of a team of Rutgers University research scientists who developed the antibiotic streptomycin in 1944. The discovery of the ability of streptomycin to fight *Mycobacterium tuberculosis*, the microorganism that causes tuberculosis was a medical breakthrough in the fight of an often-fatal disease. The other all-male team scientists were awarded the Nobel Prize for Medicine for the discovery, however, the lead scientist, Professor Selman Waksan chose to exclude Dr. Bugie's name from the streptomycin patent based on their reasoning that she would "get married and have a family" so it was not necessary for her to be recognized for her work. Bugie continued her work with antimicrobial substances that advanced the development of effective antibiotic treatments.

June Almeida

June Hart Almeida was a Scottish-born immigrant to Canada who, although she never completed her formal undergraduate education, became a lab technician who gained professional recognition for her skills and techniques of identification of microscopic particles. Following her return to the United Kingdom to take a position at a London medical school, Almeida used antibodies to pinpoint viral particles. The antibodies from previously infected individuals were introduced into samples and were drawn to their antigen-counterparts, congregating around the viruses, making them visible under the microscope and opening up a new way to diagnose viral infections in patients. In 1964, using the electron microscope, June Almeida was the first to identify the coronavirus, the pathogen we are well-familiar with after the COVID-19 pandemic.

A Century of Scientific Breakthroughs in Microbiology

As the twentieth century progressed, discoveries in microbiology, bacteriology, virology, and genetics seemed to develop exponentially and have continued into the twenty-first century. Some of the notable discoveries, advances, and events in microbiology during the past 120 years include the following timeline.

1902: Cambridge, Massachusetts enacted a mandatory smallpox vaccination program following an outbreak of the disease. Following a failed challenge to the city health department's mandate by an individual who refused to be vaccinated, the case was heard by the U.S. Supreme Court which ruled in 1905 that the state of Massachusetts had the right to make vaccination compulsory as a public health protection against communicable disease.

1904: William Gorgas brought mosquito control methods to construction sites of the Panama Canal including netting, screens, fumigation, and draining of stagnant water. The following year, the last case of mosquito-borne yellow fever was reported in Panama City, Panama.

1905: The last case of the yellow fever epidemic in North America was reported in New Orleans, Louisiana.

1905: Polio, also known as infantile paralysis, was reported to be a contagious disease transmitted from person-to-person contact following epidemic in Sweden.

1906: The first diphtheria antitoxin was produced by Ernst Lederle who founded Lederle Laboratories which later became part of Wyeth Laboratories.

1906: Belgian scientists Jules Bordet and Octave Gengou were the first to isolate *Bordetella pertussis* responsible for pertussis, also known as whooping cough.

1907: Mary Mallon, later given the name "Typhoid Mary" was placed in forced confinement when investigators found that her employment history as a cook had exposed numerous individuals who contracted typhoid fever and at least two died. The "healthy carrier" concept of disease transmission was recognized and accepted. Though she was released in 1910 with the promise to not work as a cook, Mary used a pseudonym and began to work at a hospital as a cook and was again identified as the probable infective carrier when numerous patients contracted typhoid fever. She was again forced into **quarantine** on North Brother Island in New York until her death in 1938.

1908: Karl Landsteiner, MD and Erwin Popper, MD determined that a virus is the cause of polio. The physicians from Vienna used cerebrospinal fluid of a patient who died from polio, passed it through special filters, and injected into a laboratory monkey which subsequently developed the infection.

1910: Paul Ehrlich published his findings of a successful treatment for syphilis in the November issue of *The Journal of Cutaneous Diseases*.

1913: Bela Schick produced a widely used "Schick test"—a skin test that shows whether an individual is susceptible or immune to diphtheria. A massive immunization program for those who tested positive resulted in a dramatic decrease in diphtheria cases.

1918: In March, 46 soldiers at Fort Riley, Kansas, died from an outbreak of influenza. With the onset of World War I, soldiers from Ft. Riley were deployed to fight in Spain. Soon, troops from all of the countries involved in the conflict came down with the same disease. Soldiers returned to the United States as well as to the other countries. What was later named the Spanish Flu Pandemic of 1918 spread worldwide and killed an estimated 20–50 million. The combined official death toll of WWI was 16 million. One in four people in the United States was afflicted with the virulent mutated flu virus.

1928: Scottish bacteriologist Alexander Fleming accidentally discovered a mold later identified as *Penicillium notatum* while throwing out contaminated Petri dishes. He noticed that there was a clear line of demarcation between the mold and where the bacteria had stopped growing. Fleming was responsible for naming the secretion of the mold **penicillin**, the first **antibiotic**.

1933: Rebecca Lancefield proposed a system for classifying streptococci based on how antigens in the walls of cells reacted with the human immune system. She was able to classify streptococci into various serotypes.

1937: The disease West Nile virus was first documented in Uganda. It resulted in fatal encephalitis in humans.

1942: A covert Japanese program called Unit 731 performed horrific experiments on human subjects, exposing them to anthrax, cholera, typhus, and bubonic plague to perfect **germ warfare** tactics. It is believed that the unit was responsible for releasing insects coated with these diseases from war planes flying over provinces in China over several years. An estimated 270,000 were victims of the attacks.

1943: During WWII in Europe, one million diphtheria cases, with 50,000 deaths, accompanied other disruptions of life during the war and disruption in Europe.

1944: Oswald Avery, Colin MacLeod, and Maclyn McCarty confirmed that deoxyribonucleic acid (DNA) is the carrier of hereditary information.

1945: The first influenza vaccines using inactivated influenza A and B strains were developed by Dr. Thomas Francis, Jr. and Dr. Jonas Salk and approved for military use. A year later, it was released for use by the public.

1946: Joshua Lederberg and Edward Tatum discovered the process of **conjugation**, in which the genetic material from one bacterium could be transferred to another.

1947: The first case of penicillin-resistant *Staphylococcus aureus* was reported.

1949: A Texas woman who had the last reported case of smallpox in the United States died.

1953: James Watson and Francis Crick established the model for the double helix structure and replication of DNA.

1954: Dr. Jonas Salk began a mass vaccination campaign against poliomyelitis, a viral attack of the central nervous system that could cause paralysis and asphyxiation, for children in Philadelphia. By 1955, the vaccinations were being given nationwide.

1955: Dr. Thomas Peebles was first to isolate the measles virus at Boston Children's Hospital from an infected 13-year-old student.

1958: The Food and Drug Administration (FDA) fast-tracked approval of a compound later called vancomycin to treat growing numbers of penicillin-resistant *S. aureus.*

1960: Interferon was discovered. Interferon is manufactured by specific cells of the immune system of the human body. Interferon "interferes" with the ability of viruses to replicate.

1960: Methicillin, a new antibiotic to fight resistant *S. aureus*, was released for use.

1961: Jacques Monod and Francois Jacob discovered messenger ribonucleic acid (mRNA); the chemical involved in the process of protein synthesis.

1961: First cases of methicillin-resistant *S. aureus* (MRSA) were documented.

1963: Measles vaccine developed in 1958 was widely distributed to the public.

1967: First cases of the Marburg hemorrhagic virus were reported. Laboratory workers in Marburg, Germany, were exposed while performing polio experiments with Ugandan monkeys.

1969: US President Richard Nixon announced an unconditional renunciation of biological weapons.

1971: First vaccine against meningitis was developed.

1971: The United States discontinued routine smallpox vaccination programs due to eradication of the disease.

1976: An epidemic of swine flu broke out in an Army base in New Jersey. A nationwide vaccination program prevented further spread; however, the vaccines were linked to cases of paralysis so they were discontinued by year's end.

1976: Legionnaire's disease broke out in a hotel in Philadelphia. The cause was identified as *Legionella pneumophila*, which contaminated the hotel's ventilation system.

1976: Zaire (Congo), Africa—280 people died of the Ebola virus, a hemorrhagic fever that prevents clotting.

1979: Anthrax spores leaked from a germ warfare plant in Sverdlovsk, Russia, causing more than 100 deaths in the surrounding town over a 2-month period.

1979: Australian Dr. J. Robert Warren first identified *Helicobacter pylori* in biopsy specimens of the lower stomachs of patients. In 1982, Dr. Barry Marshall grew the slow-growing bacterium in cultures.

1979: Acquired immunodeficiency syndrome (AIDS) was diagnosed for the first time. The chief sign and associated disease was Kaposi's sarcoma lesions visible on the skin of infected and dying patients.

1984: French researchers identified the human immunodeficiency virus (HIV) as the causative agent of AIDS. Robert Gallo, a US scientist, was also credited with research findings of workers in his laboratory around the same time, so credit is generally shared between the two countries. In the late 1970s, Gallo had discovered the first retrovirus, human T-cell leukemia virus (HTLV).

1987: The FDA approved the sale of AZT for treatment of HIV and AIDS. They also approved the antibiotic Cipro.

1988: Vancomycin-resistant enterococcus (VRE) was first reported in Europe. The potent antibiotic Vancomycin had been in use since 1958 as treatment for Gram-positive bacteria such as *Clostridium difficile*.

1989: The hepatitis C virus was first documented.

1995: The FDA approved the first chicken pox vaccine.

1996: The World Health Organization (WHO) warned of a growing number of highly resistant tuberculosis infections in South Africa.

1997: The CDC began work on a vaccine for avian "bird flu" virus (H5N1) after deaths in Hong Kong and fear of possible spread.

2001: Letters laced with anthrax were mailed to the New York Post and NBC, and later to several US Senators and Congressmen. Office workers who had come into contact with the letters contracted cases of cutaneous anthrax, and several US postal workers died from inhalation anthrax. **Bioterrorism** had become a reality in the United States. A governmental scientist was eventually charged with the crimes.

2002: Findings published, which were later found to be erroneous, proposed a link between childhood vaccinations and risk of autism. Parents began refusing vaccinations for their children, despite evidence of the tainted research, and cases of childhood diseases mostly eradicated began to rebound over the following years.

2002: Concerns grew over cases of Cipro-resistant gonorrhea and erythromycin-resistant group A streptococci.

2002: Two cruise ship lines dealt with outbreaks of gastrointestinal illness among large numbers of passengers.

2002: Vancomycin-resistant strains of *Staphylococcus* were identified.

2003: Cases of severe acute respiratory syndrome (SARS) began to appear in Hong Kong, Vietnam, and parts of China.

2003: MRSA was now being spread to healthy individuals through skin contact.

2003: A new strain of "mad cow disease" was identified in a young bull in Japan.

2005: French and South African researchers reported that circumcision reduced the risk of AIDS by 70 percent.

2005: Two cows in the United States died from mad cow disease, as did one in Austria.

2006: The number of cases of blindness caused by a rare fungal infection in persons who wore contacts and used commercial saline solutions increased to 122.

2006: Consumers were warned about eating spinach after 173 people became sickened by *Escherichia coli* traced back to a farm in California.

2006: The CDC warned travelers to Africa and Asia of a mosquito-borne disease, Chikungunya fever, which has symptoms similar to Dengue fever, including severe headaches, muscle pain, and joint swelling that may take months to resolve. The disease has been found in the intervening years in the islands of the Caribbean and even in the United States in 2014.

2006: *Coccidiomycosis*, better known as valley fever in California, infected 5,500 people and resulted in 33 deaths. Spores spread by disturbed soil in the Southwest were found to be the origin of the epidemic.

2006: The FDA approved a vaccine for Human Papillomavirus (HPV).

2007: An attorney from Atlanta ignored warnings about exposure of the public when he traveled to Italy by airline, despite being aware that he had a dangerous multi-drug-resistant form of tuberculosis. Government officials in the United States and Italy tried to advise all passengers of their exposure risk. Upon his return, he was forcibly quarantined in the first such action since the 1960s.

2009: A former Army nurse anesthetist pleaded guilty to assault after having infected 15 patients with hepatitis C (HCV). During surgical procedures, he would inject narcotic drugs himself and reuse the contaminated needles on patients, thus passing on his infected blood to patients in his care.

2009: A surgical technologist in Colorado exposed nearly 6,000 patients and infected 26 with HCV by stealing anesthesia narcotics and replacing them with contaminated syringes of saline.

2009: From the initial outbreak in Mexico in April through December, the CDC and WHO reported that more than 10,000 people died from and approximately 200,000 were infected with the H1N1 "swine flu" virus pandemic. In mid-June 2010, the death toll had increased to more than 18,000.

2010: Scientists in Britain showed concern about cases of patients returning from hospitals in South Asia or India with an antibiotic-resistant "superbug" called New Delhi metallo-lactamase-1 (NDM-1). One patient had died of the infection, spurring concerns about possible spread. Later, a Japanese patient treated in India also became infected with the same disease.

2010: A cholera epidemic in Haiti spread following the devastating earthquake in January that left much of the population homeless and living in squalor. In December, the death toll had reached more than 2,000, with more than 80,000 having suffered with the disease. The strain of cholera was traced back to infected United Nations troops from Nepal who had come to help with disaster relief and was spread through poorly designed sanitation facilities that contaminated the Artibonite River with human waste.

2011: US scientists cited an increase of 225 percent in oral cancers, mainly in white men between 1974 and 2007. The evidence pointed strongly to human papillomavirus (HPV) as the likely cause of the cancer increases.

2011: An Australian anesthesiologist infected 50 female patients with hepatitis C at an abortion clinic.

2011: The WHO determined that the strain of *E. coli* responsible for nearly 50 fatalities was a new variant not seen before.

2011: Health officials reported three deaths from a "brain-eating" disease, *Naegleria fowleri*, an amoeba found in water. The CDC stated that there had been 120 cases, mostly fatal since the amoeba was identified in the 1960s.

2011: An outbreak of *Listeria* found in cantaloupe from Colorado killed 33 in the United States.

2011: There were 8.7 million new cases of tuberculosis (TB) reported during the year, with 400,000 being multi-drug-resistant strains.

2012: Middle East Respiratory Syndrome (MERS), a disease similar to SARS, was identified in a Saudi Arabian man who died from severe pneumonia-like symptoms and renal failure.

2012: Seventeen people in Texas died from West Nile virus, a mosquito-borne illness.

2012: Park rangers in Yosemite closed cabins after six people became sickened and three died from hantavirus, a disease carried by deer mice and disseminated through their urine and feces.

2012: The death toll increased to 19 for patients at the National Institutes of Health (NIH) infected with an antibiotic-resistant strain of *Klebsiella pneumonia*. The outbreak was apparently attributed to a single individual.

2012: Hundreds of patients who had been given steroid injections for back pain were warned of the fungal contamination of the medication, which resulted in 30 deaths and 419 cases of meningitis. The fungus was identified as *Exserohilum rostratum.* A compounding pharmacy in Massachusetts was found to be the sole distributor of the tainted steroids but had distributed them to at least 18 states.

2012: A traveling medical technician was charged with having infected at least 39 patients with hepatitis C through stolen drugs and syringes.

2013: British officials revealed a 25 percent increase in antibiotic-resistant gonorrhea.

2013: Britain's Health Minister warned that the emergence and worldwide spread of antibiotic-resistant diseases may pose a catastrophic threat to patients in health-care settings as well as the general population.

2014: Two cases of MERS were diagnosed in patients returning from the Middle East. Since its discovery in 2012 there have been 538 cases, with 145 deaths in 17 countries.

2014: A study of healthy placentas showed a potential link between microbes typically found in the mouth to those found in placentas, in opposition to the previously held belief that the fetus grows in a sterile environment. Preliminary results may point to a benefit to the placenta from these microbes, possibly even in preventing pre-term labor.

2014: The Centers for Disease Control and Prevention in Atlanta revealed that at least 84 laboratory workers might have been inadvertently exposed to live anthrax bacteria, sparking a US Congressional investigation regarding standardization of laboratory procedures and oversight.

2014: Thousands of people were infected with Ebola hemorrhagic fever (EHF) in Guinea, Sierra Leone, and Liberia, Africa. Since its discovery in 1976, there have been 18 outbreaks of Ebola infections and a total of 11,193 fatalities through June 2015. Two nurses contracted the disease in Dallas, Texas, while treating a patient infected with Ebola while visiting Africa who died of the disease in October.

2015: Measles spread quickly in 24 states following a December 2014 outbreak originating in Disneyland in California with 117 cases linked to that source and a total of 178 cases by June 2015. California passed a mandatory vaccination law in response to the outbreak.

2015: MERS in South Korea caused shutdowns of schools and large quarantines following 166 cases through June.

2016: The World Health Organization (WHO) Emergency Committee announced a strong association between Zika virus infection in pregnant women and microcephaly and other birth defects in their infants. The virus is spread by mosquitos and was found to be transmissible by human sperm. Approximately 600,000 cases were recorded in North, Central, and South America.

2018: CDC's PulseNet that includes public health departments in all 50 states was able to use whole-genome sequencing for subtyping pathogens that cause foodborne illness including: *Salmonella, Yersinia, Vibrio, Shigella, and Cronobacter*.

2019: The Centers for Disease Control and Prevention (CDC) reported 704 cases of measles in the U.S., the largest number of cases in a single year since 1994.

2019: On December 31, health officials from Wuhan in China's central Hubei province confirmed an outbreak of dozens of pneumonia cases from an unknown pathogen.

2020: Public health agencies responded in January to the outbreak caused by a novel coronavirus first identified in Wuhan. The WHO subsequently gives the new SARS-CoV-2 disease the name COVID-19.

2020: On March 11, 2020, the WHO declared COVID-19 a world-wide pandemic. China began administering the first vaccine trials to volunteers.

2020: In March and April, the U.S. began administering vaccine trials in two doses.

2020: In August, the African continent was declared free of wild poliovirus although a small number of vaccine-derived polio infections persist.

2020: On December 8, 2020, the U.S. reached the 15 million case total. On December 8, a British 90-year-old woman became the first person in the world to receive a clinically approved vaccine. On December 11, the U.S. FDA approved use of the Pfizer vaccine for emergency use and the rollout began a few days later. The Moderna vaccine was approved for emergency use on December 18.

2021: According to the Johns Hopkins Coronavirus Research Center, by mid-May, the global number of COVID-19 infections reached nearly 163 million with 3.4 million deaths. The U.S. recorded 33 million cases with nearly 590,000 deaths. The vaccination rate skyrocketed on a global scale to nearly 1.5 billion doses given and, in the U.S., nearly 270 million doses given.

2021: Concerning COVID-19 variants from the United Kingdom, South Africa, and Brazil spread globally, worrying researchers about the effectiveness of current vaccines against mutations.

Learning from the Past

The value in examining the preceding historic timeline is not in the useless memorization of dates and facts, but rather to recognize the enormous strides that have been made in science in a relatively short period and to see how interconnected we are as individuals to the rest of humanity and the planet on which we live. One brief century after the 1918 Spanish Flu global pandemic, the world was again caught off guard by the COVID-19 pandemic. Each of us will remember the impact of the pandemic going forward but it raises the question of how much did we learn on an individual, societal, or global health scale about personal and collective responsibility, critical disaster planning for future outbreaks, and the fragility of the human species?

The microbial world is incredibly resourceful in its survival methods and, for good or for bad, the human race must find a way to coexist with it. As the relative size of the planet shrinks through global travel and access, the expanding problems and diseases that plague other countries now find their way onto our own doorsteps and from our shores to theirs. These shared experiences should unify us and magnify our need to work collectively as a species to be just as resourceful as the innumerable members of the natural microbial world to ensure our survival.

Breaking the Chain of Disease Transmission

There are many ways by which pathogenic microorganisms can be transmitted. In the timeline of the previous section, some examples of how diseases can spread include:

- Human to human
- Animal to human
- Environment to animal or human
- Insect to animal or human
- Laboratory specimens to human
- Bioterrorist attacks with various dissemination methods

Transmission of disease cannot always be prevented; however, those who work in healthcare have tools at their disposal that allow them to simultaneously protect themselves from exposure to patients and protect patients from exposure to personnel (see Figure 1-9). These tools include use of personal protective equipment (PPE) including gowns, gloves, masks, shoe covers, goggles/ face shields, N-95 respirators, aprons, etc. as well as sets of guidelines that guide their professional work practices. The COVID-19 pandemic

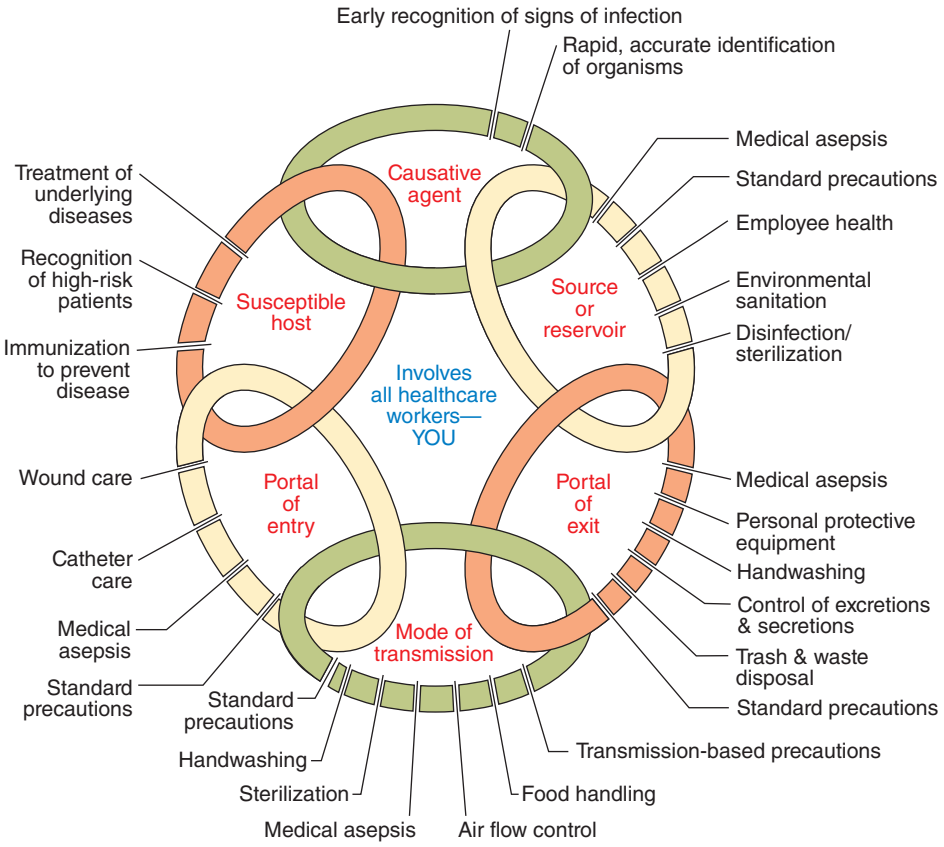


Figure 1-9 Components of the chain of infection with ways it can be broken.

brought a never-before-seen focus on the need for massive amounts of PPE during a global pandemic response and how critical but fragile that supply chain can be when not maintained or managed effectively.

Standard Precautions

An awareness of diseases and their transmissibility is a good start; however, without definitive guidelines that outline professional best practices for everyone working within the various healthcare settings, consistency cannot be achieved (see Figure 1-10).

A special section within the Centers for Disease Control and Prevention (CDC) is the Healthcare Infection Control Practices Advisory Committee (HICPAC). The members of this committee are charged with making and reviewing guidelines, and advising the CDC, the US Secretary of Health and Human Services (HHS), and other agencies regarding a broad scope of public health issues. In Section III.A of the *2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings which was updated in 2019*, the HICPAC members state "Standard Precautions combine the major features of Universal Precautions (UP) and Body Substance Isolation (BSI) and are based on the principle that all blood, body fluids, secretions, excretions except sweat, non-intact skin, and mucous membranes may contain transmissible infectious agents. Standard Precautions include a group of infection prevention practices that apply to all patients, regardless of suspected or confirmed infection status, in any setting in which healthcare is delivered. These include: hand hygiene; use of gloves, gown, mask, eye protection, or face shield, depending on the anticipated exposure; and safe injection practices. Also, equipment or items in the patient environment likely to have been contaminated with infectious body fluids must be handled in a manner to prevent transmission of infectious agents (e.g., wear gloves for direct contact, contain heavily soiled equipment, properly clean and disinfect or sterilize reusable equipment before use on another patient)."

In Section III.A.1, they include additional measures to augment the standards and state,"Infection control problems that are identified in the course of outbreak investigations often indicate the need for new recommendations or reinforcement of existing infection control recommendations to protect patients. Because such recommendations are considered a standard of care and may not be included in other guidelines, they are added here to Standard Precautions. Three such areas of practice that have been added are: Respiratory Hygiene/Cough Etiquette, safe injection practices, and use of masks for insertion of catheters or injection of material into spinal or epidural spaces via lumbar puncture procedures (e.g., myelogram, spinal or epidural anesthesia). While most elements of Standard Precautions evolved from Universal Precautions that were developed for protection of healthcare personnel, these new elements of Standard Precautions focus on protection of patients."

Transmission-Based Precautions

Standard Precautions are used in all circumstances and presume that all patients are potential carriers of undiagnosed disease. When a specific disease process or pathogenic condition has been identified, additional work practices are used to address the method by which it is spread. The (July 2019 updated) 2007 HICPAC report states in Section III.B,"There are three categories of Transmission-Based Precautions: Contact Precautions, Droplet Precautions, and Airborne Precautions. Transmission-Based Precautions are used when the route(s) of transmission is (are) not completely interrupted using Standard Precautions alone. For some diseases that have multiple routes of transmission (e.g., SARS), more than one Transmission-Based Precautions category may be used. When used either singly or in combination, they are always used in addition to Standard Precautions."

In the six listed developments included in the Executive Summary of the updated HICPAC report, only months before the beginning of the COVID-19 outbreak, Section 2 stated "The emergence of new pathogens (e.g., SARS-CoV associated with the severe acute respiratory syndrome [SARS], Avian influenza in humans), renewed concern for evolving known pathogens (e.g., *C. difficile*, noroviruses, community-associated MRSA [CA-MRSA]), development of new therapies (e.g., gene therapy), and increasing concern for the threat of bioweapons attacks, established a need to address a broader scope of issues than in previous isolation guidelines." As if almost foreseen for the year ahead (2020), in Section 3 the report states, in part, "The need for a recommendation for Respiratory Hygiene/Cough Etiquette grew out of observations during the SARS outbreaks where failure to implement simple source control measures with patients, visitors, and healthcare personnel with respiratory symptoms may have contributed to SARS coronavirus (SARS-CoV) transmission. The recommended practices have a strong evidence base." The ongoing research and lessons learned from the COVID-19 pandemic will undoubtedly reach far and wide and the delineation of best practices may be modified as a result (see Figure 1-11).

Surgical Conscience

The guiding principle under which a surgical technologist practices is called surgical conscience. A break in technique compromises surgical patient care and could threaten the patient's life. If a surgical technologist recognizes a break in technique, they must admit the break and take corrective actions. It requires vigilance in monitoring of the sterile field and addressing any breaks in technique by other team members if they occur. In the case of a break in technique that is not realized by the surgical technologist, when another team member points it out, surgical technologist should not question or argue the point and resolve the break with immediate corrective action. A surgical conscience should be imbedded in the members of the team and is the critical foundation of sterile technique. A person who does not have a strong

STANDARD PRECAUTIONS

Assume that every person is potentially infected or colonized with an organism that could be transmitted in the healthcare setting.

Hand Hygiene

Avoid unnecessary touching of surfaces in close proximity to the patient.

When hands are visibly dirty, contaminated with proteinaceous material, or visibly soiled with blood or body fluids, wash hands with soap and water.

If hands are not visibly soiled, or after removing visible material with soap and water, decontaminate hands with an alcohol-based hand rub. Alternatively, hands may be washed with an antimicrobial soap and water.

Perform hand hygiene:

- Before having direct contact with patients.
- After contact with blood, body fluids or excretions, mucous membranes, nonintact skin, or wound dressings.
- After contact with a patient's intact skin (e.g., when taking a pulse or blood pressure or lifting a patient).
- If hands will be moving from a contaminated-body site to a clean-body site during patient care.
- After contact with inanimate objects (including medical equipment) in the immediate vicinity of the patient.
- After removing gloves.

Personal protective equipment (PPE)

Wear PPE when the nature of the anticipated patient interaction indicates that contact with blood or body fluids may occur.

Before leaving the patient's room or cubicle, remove and discard PPE.

Gloves

Wear gloves when contact with blood or other potentially infectious materials, mucous membranes, nonintact skin, or potentially contaminated intact skin (e.g., of a patient incontinent of stool or urine) could occur.

Remove gloves after contact with a patient and/or the surrounding environment using proper technique to prevent hand contamination. Do not wear the same pair of gloves for the care of more than one patient.

Change gloves during patient care if the hands will move from a contaminated body-site (e.g., perineal area) to a clean body-site (e.g., face).

Gowns

Wear a gown to protect skin and prevent soiling or contamination of clothing during procedures and patient-care activities when contact with blood, body fluids, secretions, or excretions is anticipated.

Wear a gown for direct patient contact if the patient has uncontained secretions or excretions.

Remove gown and perform hand hygiene before leaving the patient's environment.

Mouth, nose, eye protection

Use PPE to protect the mucous membranes of the eyes, nose and mouth during procedures and patient-care activities that are likely to generate splashes or sprays of blood, body fluids, secretions and excretions.

During aerosol-generating procedures wear one of the following: a face shield that fully covers the front and sides of the face, a mask with attached shield, or a mask and goggles.

Respiratory Hygiene/Cough Etiquette

Educate healthcare personnel to contain respiratory secretions to prevent droplet and fomite transmission of respiratory pathogens, especially during seasonal outbreaks of viral respiratory tract infections.

Offer masks to coughing patients and other symptomatic persons (e.g., persons who accompany ill patients) upon entry into the facility.

Patient-care equipment and instruments/devices

Wear PPE (e.g., gloves, gown), according to the level of anticipated contamination, when handling patient-care equipment and instruments/devices that are visibly soiled or may have been in contact with blood or body fluids.

Care of the environment

Include multi-use electronic equipment in policies and procedures for preventing contamination and for cleaning and disinfection, especially those items that are used by patients, those used during delivery of patient care, and mobile devices that are moved in and out of patient rooms frequently (e.g., daily).

Textiles and laundry

Handle used textiles and fabrics with minimum agitation to avoid contamination of air, surfaces and persons.

SPR

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Figure 1-10 Standard Precautions.

Debora Cartagena/CDC

Figure 1-11 N-95 respirator masks.

surgical conscience should not work in an operating room and poses a risk to their own health and, more importantly, to the patients who come into the surgical environment of care.

Aseptic and sterile techniques, as well as methods of disinfection and sterilization, are discussed further in later sections.

MICRO NOTES

"A Breath of Fresh Air"

Did you know that the line of mouthwash products called Listerine® were actually named for a famous physician? The company site states, "It begins back in 1879, when LISTERINE® Antiseptic was first formulated by Dr. Joseph Lawrence and Jordan Wheat Lambert. It was named after Dr. Joseph Lister, who was the first person to perform an antiseptic surgery. His work paved the way for the modern operating room and lead to significant decline in patient mortality in the nineteenth century. In the early days, claims about the use of LISTERINE® Brand ranged widely, from curing sore throats and colds to healing wounds, but its germ-killing power has never faded." Now you know, and you can thank Dr. Lister for that oh-so-fresh breath you get after you gargle, swish, and rinse!

Under the Microscope

Microbiology research and its application to healthcare have been studied for centuries. The use of traditional tools of Gram staining and Koch's Postulates and subsequent advanced tools such as the electron microscope over the past century have brought the impact of the microbial world on human health into clear focus. The Centers for Medicare and Medicaid Services (CMS) have initiated revised policies regarding reimbursement to hospitals for care of patients who suffer healthcare-associated infections (HAIs) such as SSIs. Hospitals must bear the costs of treatment of preventable infections acquired in the course of their interaction with the medical profession which has the responsibility to utilize the scientific foundation built by the experimental trials and errors of historical, recent, and current pioneers in the war against disease and human suffering. Healthcare workers, including surgical team members, are one critical key in preventing patient injury and protecting hospitals.

1. What are examples of a routine procedures performed by surgical technologists and other surgical team members prior to entering the sterile field that would be part of aseptic technique?
2. List components of personal protective equipment (PPE) that serve as barrier protection for patient and personnel interactions and correlate the various components applicable to the procedures being performed.
3. Which vaccinations are required for personnel in the operating room and why?
4. Which historical figures in medicine are credited with recognizing the need for aseptic techniques to reduce wound infections?
5. Which set of measures are used in addition to Standard Precautions when the disease status of a surgical patient has been determined in advance?

CHAPTER 2

The Science of Microbiology

Learning Objectives

After completing the study of this chapter, you will be able to:

1. Define key terms.
2. Distinguish between normal flora and pathogenic microbes.
3. Compare the various classification systems of living organisms.
4. Discuss characteristics of the prokaryotes.
5. Describe how viruses and prions become pathogenic.
6. Discuss the systems of nomenclature and taxonomy to classify microbes.
7. Apply critical thinking skills in relating chapter material to the surgical environment of care or broader global community.

Key Terms

Acellular
Archaea
Capsid
Capsomere
Cladistics
Creutzfeldt-Jakob disease (CJD)
Eukaryotes
Genome
Microbiology
Microbiome
Morphology
Normal flora
Osmotrophic
Pathological condition
Phenetics
Phylogeny
Prions
Prokaryotes
Protoplasm
Retrovirus
Surgical site infection (SSI)
Taxonomic hierarchy
Taxonomy
Transmissible spongiform encephalopathy (TSE)
Viruses

The Big Picture

Surgical technologists are keenly aware of the existence of microbial life in the surgical environment and even on our own skin. Transient microbes also known as microflora are the temporary passengers that we each acquire as we touch surfaces or that deposit on our skin or hair from the environment around us. Our resident or indigenous microbes are normal microflora that live deep in our skin layers. The same holds true for the patient's skin. Invasive procedures require that the skin is cleansed with antimicrobial soap prior to the incision.

During your examination of the topics in this chapter, consider the following:

1. How do we, as surgical technologists, deal with our own resident microbes before going into the operating room to prepare for surgery?
2. Why do we go to such lengths if microbes are everywhere?
3. Why is it important to determine the characteristics and classifications of microbes?
4. What are aseptic and sterile techniques?

Clinical
Significance
Topic

There is a saying, "In the eyes of the law, if it isn't documented, it didn't happen." The idea may be stated in other words, but the lesson is the same: scientific research and healthcare require documentation of every procedure, outcome results, steps taken, and identity of all persons involved in clinical studies or in surgical procedures. Science, like surgery, relies on critical procedural steps, accurate documentation, clear communication, professional collaboration, technical skill, and personal integrity. Standards of care are foundational principles based on those criteria. Patients rely on best practices in scientific research and in the surgical arena.

What Is Microbiology?

Encyclopaedia Britannica defines **microbiology** as, "The study of microorganisms, or microbes, a diverse group of minute, simple life forms that include bacteria, archaea, algae, fungi, protozoa, and viruses. The field is concerned with the structure, function, and classification of such organisms and with ways of both exploiting and controlling their activities." In the surgical environment of care, controlling the microbial populations encountered is of paramount importance in prevention of **surgical site infections (SSIs)**.

Overview of Microbes

Microbes, often referred to as microorganisms, are extremely small living beings or bits of material that actually may not be alive at all in the traditional sense. As their names imply, they are invisible to the naked eye and were only accepted by scientists after Van Leeuwenhoek created the microscope, began observing these tiny populations, and then recorded and published his findings. His single-lens microscope was sufficient to view some of the larger microbes he found in rainwater, saliva, and sperm. Long after that initial critical invention, much stronger magnification systems such as the electron microscope have been developed that allow scientists to explore the even more elusive world of viruses and other microbial life that would otherwise remain unseen. This chapter outlines the various forms and classifications of microbes as well as the scientific classification systems used to categorize and name them.

Ancestors of Life on Earth

Microbes are typically associated with disease, and they are the purveyors of many terrible **pathological conditions**. The reality however is that no living organism on the planet would exist or continue to survive without the smallest and most populous life forms—microbes. They can be found everywhere on Earth, including inhospitable environments such as deep inside ancient glaciers and geothermal hot springs.

Most of the millions of types of microbes on the planet pose no threat to our species. Humans and animals depend on the **normal flora** or bacterial populations of the gastrointestinal tract and skin to fend off invading or competitive pathogenic microbes. Normal flora allows our bodies to synthesize nutrients from our food while it travels through the

various regions of the gastrointestinal tract. Unfortunately, even these beneficial inhabitants can cause problems if they escape their normal environment, or **microbiome**, and become invaders themselves.

Scientists have been able to identify evidence of microbial life dating back to more than 3 billion years by some estimates, making them most likely the first living things on the planet. Microbes are able to adapt to their environment to ensure survival and certain species can even live without oxygen. Viruses and prions are examples of microbes that are not considered living organisms, but they are able to invade healthy cells and tissues and spread through the host's normal cellular division and growth.

Scientific Classifications of Microbes

Since ancient times, humans have devised ways in which to classify the things around them. In the fourth century BC (BCE), the Greek philosopher Aristotle wrote about a classification system of living beings based on observations of the similarities and differences between species. His system had two components referred to as kingdoms: plantae (plants) and animalia (animals). It was many centuries later before anyone modified this long-accepted system.

Ernst Haeckel, a German biologist, added a third class in 1894 as the scientific community explored microorganisms. His addition to the kingdoms of plantae and animalia was the kingdom Protista. This group included single-cell **eukaryotes** and bacteria (**prokaryotes**).

In 1956, American biologist Herbert Copeland split the kingdom Protista that Haeckel had described into two distinct parts, thereby adding a fourth kingdom: bacteria. He observed that there were enough substantial differences between single-cell eukaryotes and bacteria that it merited creating a new separate kingdom.

Robert Whittaker, an American plant ecologist, was the next to revise the kingdom classification system. He first proposed his Five Kingdom classification in 1959, which he later refined in 1969. The Five Kingdom classification system was used widely in scientific circles and may still be found in current literature. Whittaker's classifications were:

- Plantae (plants)
- Animalia (animals)
- Protista (single-cell eukaryotes)
- Monera (single-cell prokaryotes)
- Fungi (single-cell or multi-cell **osmotrophic** eukaryotes)

In 1977, Carl Woese, an American biophysicist and evolutionary microbiologist at the University of Illinois, along with his colleagues announced the discovery of a category of microbes, distinctly different genetically from eukaryotes and prokaryotes. They called this new category **archaea**. Dr. Woese spent years studying the genetic sequences of microbial ribosomes and ribosomal DNA (protein building structures in cells). He was able to determine through this research that these archaea found in some of the harshest environments including glaciers and hot springs, shared a single genetic ancestor with the prokaryotes and eukaryotes, but that they had each evolved to be very different from one another. He initially proposed a Six Kingdom classification system in 1977, which modified Whittaker's system. He changed Monera to Eubacteria and added Archaebacteria, which was defined as including prokaryotes that were genetically different from other prokaryotes and actually more closely resembled eukaryotes.

Three Domains System of Classification

Recognizing the potential for confusion from so many versions of classification systems, Dr. Woese proposed an entirely new system in 1990—the Three Domains system. He simplified the groups and defined them as:

- Bacteria (single-cell prokaryotes)
- Archaea (prokaryotes that differ from bacteria in their genetic transcription and translation and are more similar to eukaryotes)
- Eukarya (multi-cell plants and animals; single-cell eukaryotes; single-cell and multi-cell osmotrophic eukaryotes)

The first four classification systems were devised from **phenetics**, the scientific observations of the similarities between organisms. Dr. Woese's research dealt with **cladistics** and **phylogeny**, studies that map out the evolution of a genetically related group of organisms, creating a type of genetic microbial family tree (see Figure 2-1).

In a 1996 New York Times interview, Dr. Woese said, "It's clear to me that if you wiped all multi-cellular life-forms off the face of the earth, microbial life might shift a tiny bit. If microbial life were to disappear, that would be it—instant death for the planet." He also stated that microbes accounted for more of the living **protoplasm** on Earth than every plant, animal, and human being combined.

Two Empires and Three Domains

Researchers have continued to strive for explanations to complex questions regarding the connections between all life forms on Earth. Comparative genomics called into question the accepted theory of an ancestral "tree of life" encompassing all living cells. Scientists studying nucleotide sequences for the genomes of the three domains of life, bacteria, archaea, and eukarya, theorized in 2010 that a better representation would be that of two major groups or empires, one for cellular organisms (including the three domains) and one for viruses (including plasmids, transposons, and other particles of genetic material). These researchers proposed replacing graphic models of family trees with network connections for the prokaryotes and viruses that contained complex genetic crosslinks or horizontal gene transfer. The genome researchers discovered evolutionary changes that combined traits of both bacteria and archaea and may explain the emergence of antibiotic-resistant strains of bacteria. Ultimately, genomic researchers have come to the conclusion that the empire of

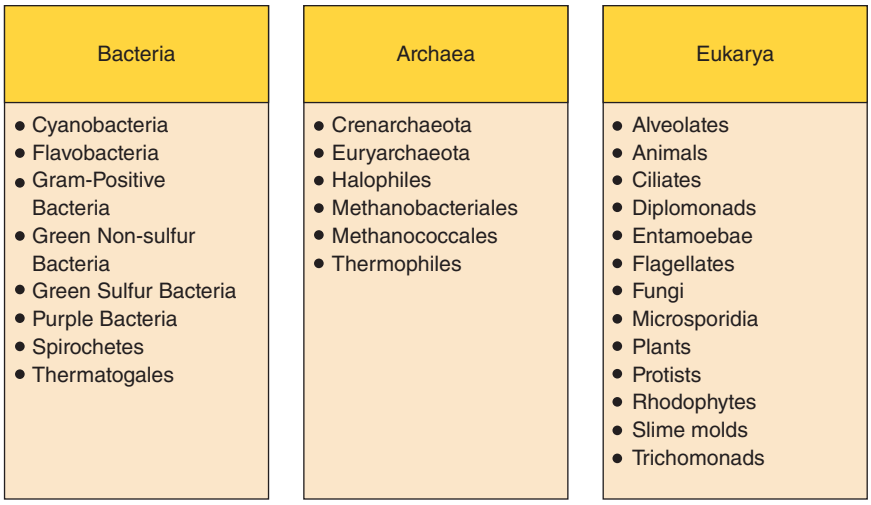


Figure 2-1 Three Domains classification of organisms.

viruses, tiny though they may be individually, far surpasses the empire of cellular life in size and diversity.

Eukaryotes

Humans, mammals, plants, birds, and insects are living organisms composed of eukaryotic cells. Each eukaryote's cells have membrane-bound organelles and a nucleus that contains deoxyribonucleic acid (DNA). Single-cell eukaryotes include protozoa, algae, and fungi. Cell structures of eukaryotic microbes are discussed further in Chapter 5.

Prokaryotes

All bacterial cells are prokaryotes. These microbes contain genetic material but they do not have a nucleus as part of their cell structure. A specific type of prokaryote is cyanobacteria. Once called blue-green algae, cyanobacteria are aquatic and photosynthetic, meaning they live in water and produce their own food. Fossils of them have been found that date back more than 3.5 billion years. The oxygen produced by photosynthesis of cyanobacteria changed the Earth's atmosphere early in the planet's history and created the more hospitable environment that allowed for life to form. Prokaryotic cell structure is discussed further in Chapter 4.

Archaea

Archaea are the newest forms of prokaryotes to be studied, but they may actually pre-date any other forms in the historical timeline of our planetary evolution. These single-cell organisms share similar cellular structure with bacteria; however, they have distinct genetic differences. Archaea show an affinity for unusually extreme environments, including glaciers, geothermal springs, and salt marshes.

Non-Living Pathogens

In addition to the eukaryotes, prokaryotes, and archaea, there are types of pathogens that do not fit the description of living microorganisms but are covered under the general heading of microbes. These special exceptions include viruses and prions.

Viruses

Viruses are extremely small bundles of genetic material, either DNA or ribonucleic acid (RNA), only visible with the use of an electron microscope. They can be 10,000 times smaller than some bacteria. They are **acellular** and consist of nucleic acid wrapped in a coating called a **capsid** made of proteins called **capsomeres**. Some viruses will also have an additional wrapping called an envelope. **Retroviruses** utilize and spread only RNA genetic material and are responsible for the disease human immunodeficiency virus (HIV). Viruses are incapable of performing reproductive functions on their own and are sometimes referred to as obligate intracellular parasites. Without a host cell that can reproduce, viruses are metabolically inert and incapable of multiplying. The empire of viruses is discussed in depth in Chapter 8.

Prion Diseases

Transmissible spongiform encephalopathies (TSEs) are found in both animals and humans and comprise a group of diseases caused by infectious agents found mainly in structures of the central nervous system, most often the brain. These agents are called **prions** (from proteinaceous infectious particles) and have no nucleic acids and no cellular structure. The mechanism of infection is thought to be an abnormal folding of these normal prion proteins that destroy brain tissue and create holes, resulting in the appearance of a sponge (spongiform). Animal TSE infections are called bovine spongiform encephalopathy (mad cow disease), ovine spongiform encephalopathy (scrapie), and chronic wasting disease (CWD) in North American hooved animals such as deer, elk, and moose.

The prion infections in humans are **Creutzfeldt-Jakob disease (CJD)** and variant Creutzfeldt-Jakob disease (vCJD), Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, and Kuru.

These diseases are discussed further in Chapter 8.

Taxonomy

The system of classifying every living organism is called **taxonomy**. The objectives of taxonomy are to:

1. Establish relationships between like organisms
2. Differentiate between two groups of organisms
3. Classify a previously unknown organism

Taxonomy is especially important in the identification of a microbe that is capable of, or actively causing a disease. The bacterium is isolated from a patient and its characteristics compared to the characteristics of microbes already classified. After the pathogen has been identified, the course of treatment can be prescribed.

The last important purpose of the science of taxonomy is that it establishes a universal language of communication used by scientists and microbiologists around the world. Taxonomy establishes a system of categories called taxa (singular, taxon) that reveal the degree of relationship among microbes; in other words, the taxa show the phylogenetic (common ancestor) relationships. It is from this foundation that scientists can communicate on a mutual, standardized basis concerning the complex world of microbes.

Nomenclature

In the eighteenth century, Carolus Linnaeus developed a scientific nomenclature that standardized the naming and classification of organisms. He used Latin names because the scholars of his time wrote in Latin.

Binomial Nomenclature

The system is referred to as binomial nomenclature. Every living organism has two names: genus name and species name. Both names are underlined or italicized when written. The genus name is always capitalized and the species name is in lowercase. Additionally, the genus name is always a noun and, typically, the species name is an adjective. In written text, the first time the name is used, it must be fully spelled out. Subsequent uses of the genus name can be abbreviated by using the first capitalized letter of the name. The species name, however, is never abbreviated. Consider the following examples:

1. *Homo sapiens* (human species); *Homo* is the genus and means "man"; *sapiens* is the species and means "wise." It is abbreviated as *H. sapiens*.
2. *Klebsiella pneumoniae* (one type of bacteria that causes pneumonia); *Klebsiella* is the genus and is derived from the name of scientist Edwin Klebs, who discovered the microbe; *pneumoniae* is the species and specifically describes the disease it causes. It is abbreviated as *K. pneumoniae.*
3. Bacteria are sometimes referred to using a shortened portion of the genus name with the species name or even just the shortened genus. Examples include *Staph aureus* and staph or strep.

The International Committee on Systematics of Prokaryotes establishes the rules for assigning a name to a new classified bacterium and to which taxa the bacterium is assigned. The rules are published in the *International Code of Nomenclature of Prokaryotes* (commonly referred to as the Bacteriological Code). The descriptions of bacteria and the evidence for their chosen classification are published in the *International Journal of Systematic and Evolutionary Microbiology*. After this has taken place, the bacterium can be placed in the most well-known and standardized reference, *Bergey's Manual of Systematic Bacteriology*, discussed later in this section.

Taxonomic Hierarchy

All living organisms are placed in a system of subdivisions that comprise what is called the **taxonomic hierarchy**. Beginning at the top of the classification is the species, closely related organisms that interbreed and are the basic grouping unit of living organisms. Next is the genus, which comprises species that are related by descent but differ from each other in particular ways. Related genera (plural of genus) comprise a family. A group of families comprise an order and a group of similar orders constitute the next group, called a class. Related classes comprise a division or phylum, and all divisions that are similar to each other constitute the last grouping, called a kingdom. Revised models of the taxonomic hierarchy include the three-domain system (archaea, bacteria, and eukarya) as well (see Figure 2-2).

In summary, the order of the divisions from largest group to smallest is as follows: kingdom, division (phylum), class, order, family, genus, and species.

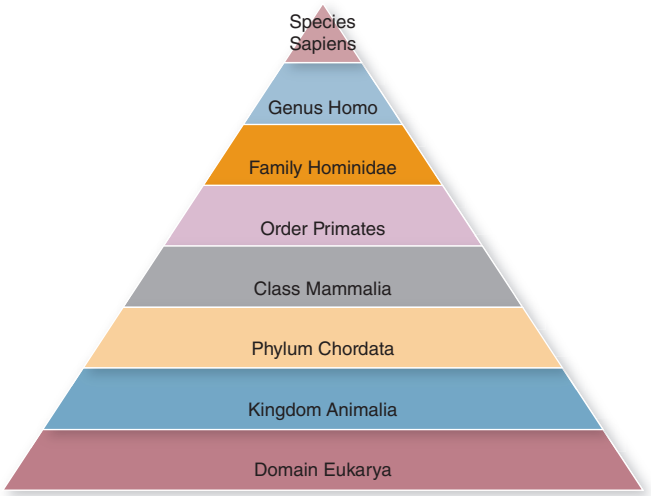


Figure 2-2 Taxonomic hierarchy of humans (including domain).

Standardized Classification of Prokaryotes

As previously mentioned, the taxonomic classification for bacteria is published in *Bergey's Manual of Systematic Bacteriology*. The first edition of the reference had four separate volumes with publication dates from 1984 to 1989. The current reference, the second edition, has five volumes focusing on the following areas:

- Volume 1 (2001) The *Archaea* and the deeply branching and phototrophic *Bacteria*
- Volume 2 (2005) The *Proteobacteria*
- Volume 3 (2009) The *Firmicutes*
- Volume 4 (2011) The *Bacteroidetes*, *Spirochaetes*, *Tenericutes* (*Mollicutes*), *Acidobacteria*, *Fibrobacteres*, *Fusobacteria*, *Dictyoglomi*, *Gemmatimonadetes*, *Lentisphaerae*, *Verrucomicrobia*, *Chlamydiae*, and *Planctomycetes*
- Volume 5 (2012) The *Actinobacteria*

Bergey's Manual of Systematic Bacteriology was preceded by the 1936 publication of *Bergey's Manual of Determinative Bacteriology*. Realizing the value of having a resource for the scientists working in the field of microbiology, the Bergey's Manual Trust was established in 1936 as a nonprofit group charged with continually reviewing and updating research materials that provide an established classification system for the identification of bacteria (prokaryotes). Information is obtained from the analyses of DNA and RNA, chemical analyses, and other laboratory tests to create the phylogenetic models. Microbiologists from around the world contribute to the research and preparation of new reference volumes.

The challenge to the publishers of the manual is keeping pace with the explosion of information submitted by researchers. By the time a volume is distributed in print, it may be seriously deficient in the most current findings of the community. In the digital age of communications, electronic versions may provide a way in which to update outdated material more expeditiously. *Bergey's Manual of Systematics of Archaea and Bacteria* (BMSAB) was made available online for the first time in 2015. The digital edition is described as an extensive reference for microbiology with over 1750 articles that provide descriptions of the taxonomy, physiology and biological properties of over 600 new species and 100 new genera of prokaryotic taxa per year and an authoritative collection of archaeal and bacterial diversity.

Viral Taxonomy

One of the most challenging areas of microbiology is that of identifying and classifying viruses. These nonliving cellular invaders are so tiny an electron microscope is necessary to visualize them. The methods of classifying viruses include **morphology**, nucleic acid type, mode of replication, the types of host organisms invaded, and type of disease they cause. Viruses invade living organisms, large and small. Three general classifications are based on the types of host cells they penetrate. Those that infect members of the animal kingdom are termed zoophaginae. If they infect plants, then they are considered phytophaginae. Viruses will even invade prokaryotes and are classified simply as phaginae (see Figure 2-3).

ICTV Viral Taxonomy

The International Committee on Taxonomy of Viruses (ICTV) is responsible for identifying and classifying viruses in the same way that the International Committee on Systematics of Prokaryotes classifies bacterial species. The committee comprises six subcommittees that are responsible for covering fungal viruses (including algae), plant viruses, invertebrate viruses, prokaryotic (including archaea) viruses, and vertebrate viruses. The sixth subcommittee manages ICTV data and websites.

The 2021 *ICTV 10th Report* lists nine groups of viruses or viral agents:

- Double strand DNA viruses (dsDNA)
- Single strand DNA viruses (ssDNA)

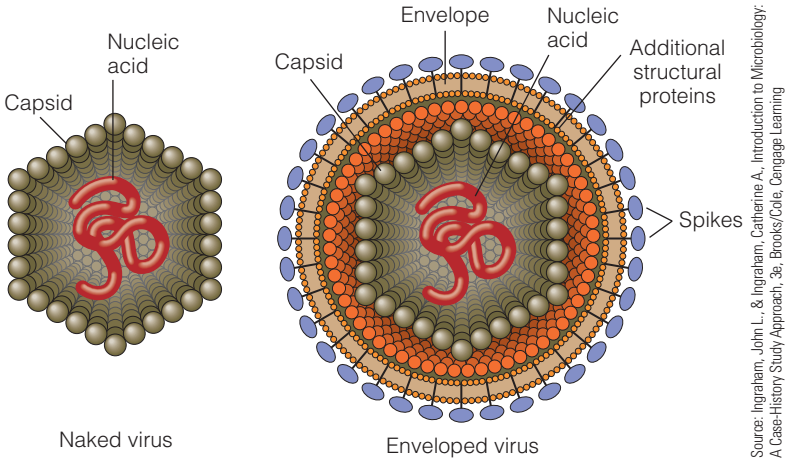


Figure 2-3 Examples of viruses.

- Single strand DNA/Double strand DNA viruses (ssDNA/dsDNA)
- Positive sense RNA viruses
- Negative sense RNA viruses
- Double strand RNA viruses (dsRNA)
- Reverse transcribing DNA and RNA viruses
- Subviral agents
- Unclassified viruses

The taxonomic hierarchy for viruses does not include domain or kingdom classifications because they are not considered living organisms, but they do have genetic codes and characteristics that allow for grouping into the taxa: order, family (or sub-family), genus, and species. The species name may be more than one word but must clearly describe the virus. For example, the HIV is a common name with subspecies given a number, such as HIV-1. The common names are not underlined or italicized. If the virus has been given a genus, then it is italicized (as in *Papillomavirus*, human wart virus, which is in the family *Papovaviridae*).

The genus name will end in the suffix *-virus.* The sub-family taxon is used when naming a complex group of genera and written with the suffix *-virinae.* The suffix used for the family taxon is *-viridae*. Order names will end in *-virales* (see Figure 2-4).

The ICTV is governed by the Virology Division of the International Union of Microbiological Societies (IUMS). In an explanation of its process of determining how to assign viral species into specific taxa on the ICTV website, they state, "As defined therein, 'a virus species is a polythetic class of viruses that constitute a replicating lineage and occupy a particular ecological niche.' A 'polythetic class' is one whose members have several properties in common, although they do not necessarily all share a single common defining property."

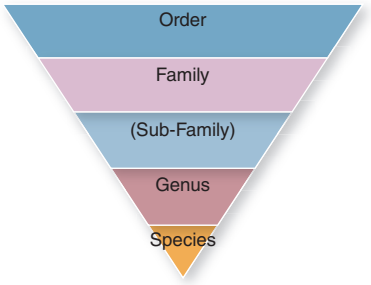


Figure 2-4 Viral taxonomic hierarchy.

Baltimore Classification System

American virologist and Nobel Laureate, David Baltimore, devised a system of classifying viruses in seven groups on the basis of the virus' **genome** and messenger RNA (mRNA). The viruses will carry either single or double strands of genetic strands of DNA or RNA. In addition, the Baltimore classification takes into consideration whether there is a positive (+) or negative (–) sense component. This designation has to do with the ability of the viral mRNA to interact with the host cell's cytoplasm and through a process of translation, creating the proteins and enzymes necessary for replication of its genome. The chapters listed in the previously discussed *ICTV 10th Report* follow this classification system.

The seven groups of the Baltimore classification of viruses are:

- Group I: Double-stranded DNA (dsDNA)
- Group II: Single-stranded DNA (ssDNA)
- Group III: Double-stranded RNA (dsRNA)
- Group IV: Positive-sense single-stranded RNA [(+)ssRNA]
- Group V: Negative-sense single-stranded RNA [(–)ssRNA]
- Group VI: Reverse-transcribing diploid single-stranded RNA (ssRNA-RT)
- Group VII: Reverse-transcribing circular double-stranded DNA (dsDNA-RT)

MICRO NOTES

"The Good Old Boys (and Girls) Club"

According to asm.org, "The American Society for Microbiology is the oldest and largest single life science membership organization in the world. Membership has grown from 59 scientists in 1899 to more than 30,000 members today, with more than one-third located outside the United States. The members represent 26 disciplines of microbiological specialization plus a division for microbiology educators."

Under the Microscope

Dev, a student taking the microbiology course pre-requisite to enter the surgical technology program, was intrigued by a surgery-related article. As neurosurgery was Dev's favorite surgical specialty, he chose this topic for his research paper to discuss the connection between microbiology and his future profession. The case in the article involved a craniotomy for biopsy in which the neurosurgeon advised the staff that the patient was being assessed for symptoms of ataxia (loss of muscle coordination) and relatively sudden onset of dementia. Tumors and any other intracranial lesions had been ruled out by radiographic studies. The patient had lived in England approximately 10 years ago when there had been an outbreak of "mad cow" disease.

1. What condition or disease process might the neurosurgeon suspect based on the patient's history as given?
2. Using binomial nomenclature and taxonomy taught in the microbiology course, how would Dev describe the other groups of organisms affected by this particular type of pathogen and general category of disease?
3. What type of infective agent is the cause for this form of transmissible spongiform encephalopathy?
4. Which scientific microbial classification system (if any) would contain this type of pathogen?
5. How would Dev describe the characteristics of the infective agents in this case as compared to the other classes of microbes studied in his course?

CHAPTER 3

The Microbiology Laboratory

Learning Objectives

After completing the study of this chapter, you will be able to:

1. Define key terms.
2. Describe the parts of a compound light microscope as discussed in the chapter.
3. Describe other types of microscopes used in a hospital or research microbiology laboratory.
4. Describe various forms of culture media as outlined in the chapter.
5. Describe the procedure for performing a Gram stain analysis.
6. Discuss the possible findings of a Gram stain test and what they indicate.
7. Discuss the variety of laboratory studies available for classification of microorganisms.
8. Apply critical thinking skills in relating chapter material to the surgical environment of care or broader global community.

Key Terms

Acid-fast stain
Agar
Anaerobic chamber
Antibodies
Antisera
Blood agar
Chocolate agar
Condenser
Culture
Culture media
Differential media
Enriched media
Enzyme
Enzyme-linked immunosorbent assays (ELISAs)
Fastidious
Flagella staining
Hybridization
Immunofluorescence
MacConkey agar
Metric system
Mordant
Ocular lenses
Oil immersion objective
Phage typing
Plaques
Reducing media
Refractive index
Resolution
Selective media
Simple stain
Slants
Spores
Virulence

The Big Picture

Surgical technologists rarely have the opportunity to visit and explore the microbiology laboratory in the hospital. Maybe it would be interesting to arrange a "shadowing" experience for lab and surgical personnel to follow one another for a day to see what each position does. Intraoperatively, the surgeon may request cultures to be taken of tissue or fluid for testing.

During your examination of the topics in this chapter, consider the following:

1. Why are two culture tubes typically used and sent for analysis?
2. Which types of microbes might be found in different tissues/areas of the body and why?
3. How might an individual's performance of laboratory studies impact the accuracy of results?
4. Why does final determination of antibiotic sensitivity of bacteria take so long?

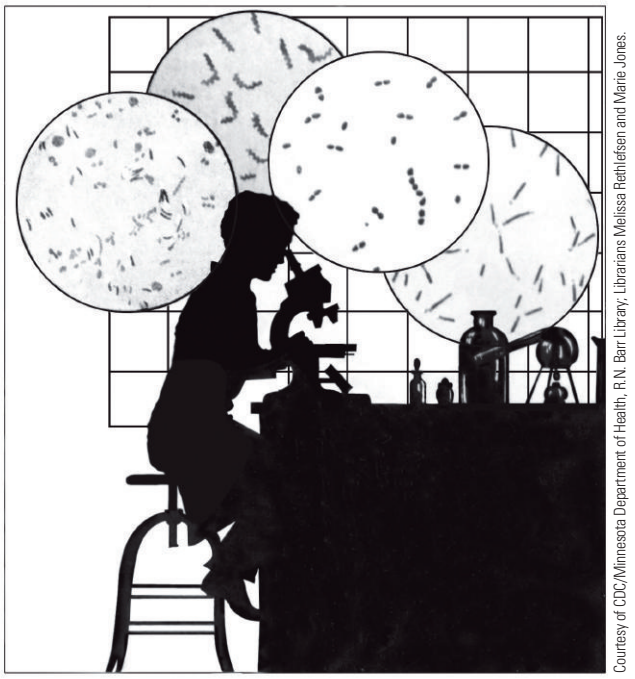
Clinical
Significance
Topic

Surgical technologists must know how to deal with specimens collected during surgery. However, to assure accurate analysis, everyone involved with the care and handling of surgical specimens must be aware of the importance and proper preparation procedures of the various types of samples sent for study. During your surgical technology education and later in your career, you will deal with frozen sections, bacterial cultures, tissue specimen staining and marking, appropriate containment of tissue, amputated limbs, foreign bodies, cancerous tumors, and bizarre anomalies. You will be making sure these specimens are properly identified, gently and appropriately handled, and prepared with (or without) fixatives. All surgical specimens must be transported to the lab in a safe and timely manner. It is a team effort that makes the difference between whether a patient is receiving or not receiving a correct diagnosis.

Microbiology Lab Personnel

The US Department of Labor Bureau of Statistics states that "microbiologists study microorganisms such as bacteria, viruses, algae, fungi, and some types of parasites. They try to understand how these organisms live, grow, and interact with their environments." The physicians and scientists are only one component of the microbiology laboratory. Just as in the operating room, there are technologists and technicians who do a large part of the day-to-day work. They function under the broad direction of research scientists, pathologists, or laboratory directors, although much of the testing and processing for which they are responsible is done largely unsupervised. This requires that these personnel be well-educated and possess the same type of moral and ethical principles that should guide all allied health professionals. Accurate diagnostic test results and reliable experimental research studies require both microbiologists and technicians to be exacting, thorough, accurate, organized, and dedicated to the scientific method (see Figure 3-1).

This chapter explores various microscopes, staining techniques, culture media, and other specialized testing methods to determine the identity and classification of microorganisms.



Courtesy of CDC/Minnesota Department of Health, R.N. Barr Library; Librarians Melissa Rethlefsen and Marie Jones.

Figure 3-1 Laboratory researcher in the 1930s.

Introduction to the Microscope

Microbes cannot be seen without the use of a microscope. The development of microbiology could not have gone beyond the advances of the nineteenth century if it were not for the invention of sophisticated microscopes that allow the smallest of organisms to be seen. This section first discusses the metric system and its use in measuring microbes.

Units of Measure

The standard unit of length in the **metric system** is the meter (m). One of the advantages of the metric system is that it is based on units of 10. The breakdown is as follows: 1 m = 10 decimeters (dm) = 100 centimeters (cm) = 1,000 millimeters (mm) (see Table 3-1).

Microbes are measured in even smaller metric units of length such as micrometers, nanometers, angstroms, and picometers. The old term micron has been replaced with micrometer and the old term millimicron has been replaced with nanometer. A micrometer (µm) is equal to 0.000001 m or 1 mm = 1,000 µm. The prefix "micro" indicates that the unit following it should be divided by one million. A nanometer (nm) is equal to 0.000000001 m (1 m = 1 billion nm) or 1 mm = 1,000,000 nm. The prefix "nano" indicates that the unit after it should be divided by one billion. An angstrom (Å) is equal to 0.0000000001 m (1 m = 10 billion Å), and 0.1 nm = 1 Å or 1 nm = 10 Å. A picometer (pm) is equal to 0.01 Å (1 Å = 100 pm) and 1 nm =1000 pm. The angstrom (Å) is no longer considered an official unit of measure, but due to its prevalent use in scientific literature it should be familiar to the student of microbiology.

Table 3-1 **Measuring Microorganisms**

Unit of Measurement	Equal To
1 meter (m)	10 decimeters (dm)
10 decimeters (dm)	100 centimeters (cm)
100 centimeters (cm)	1,000 millimeters (mm)
1 micrometer (µm)	0.000001 meter (m)
1 nanometer (nm)	0.000000001 meter (m)
1 angstrom (Å)	0.0000000001 meter (m)

Examples of sizes of microbes include the following:

1. Bacteria can range in size from 3 µm to as small as 0.2 µm.
2. Erythrocytes (red blood cells) are approximately 7 µm in diameter.
3. Many viruses range in size from 10 to 300 nm.
4. Most protozoa measure 2–200 µm in length.

Types of Microscopes

Scientists utilize various types of microscopes to visualize the microbial world. Microscopes described in this section include compound, dark-field, phase-contrast, transmission electron, and scanning electron microscopes (see Table 3-2).

Table 3-2 **Types of Microscopes**

Microscope	Special Features
Compound light	• Two-lens system with light source • High-dry lens for viewing large microbes • Oil immersion objective for viewing bacterial characteristics
Dark-field	• Used when microbes are not visible with light microscope or cannot be stained (*Treponema pallidum*) • Motility is not easily visualized
Phase-contrast	• Allows detailed visualization of internal structures of microbes • Eliminates need to fix or stain microbes
Fluorescence	• Allows visualization of naturally fluorescent microbes or those stained with fluorochromes
Electron	• Used for visualization of viruses, internal structures of cells in detail, and other objects smaller than 0.2 µm
Transmission electron	• Can resolve objects as close in proximity as 2.5 nm • Can magnify objects 10,000×–100,000×
Scanning electron	• Offers two advantages over TEM: 1. Specimen does not have to be thinly sliced. 2. Three-dimensional views are obtained.

Compound Light Microscope

The compound light or bright-field microscope is a multi-lens system combined with a light source (see Figure 3-2). The light passes through the specimen and lenses and then returns back up to the eyepiece. The condenser lens controls the light aimed at the specimen from its bottom. The objective lens is adjusted to be near the specimen from the top. The most proximal lenses to the viewer are called the **ocular lenses** and are located in the binocular eyepieces. The multi-lens compound system can magnify 40× to 2,500×. The magnification is indicated by the numeral preceded by ×, such as "1,000×," in which the × means "times."

The 40× lens is called the high-dry lens and is used to view algae, protozoa, and other large microbes. The 100× lens is the **oil immersion objective** used for viewing the unique characteristics of various bacteria. The oil immersion objective must be used with a drop of oil between the specimen and the objective lens. The immersion oil aids in reducing the scattering of light.

The light must be focused to obtain a clear view of the specimen. The **condenser**, located below the fixed stage, is used to focus light onto the specimen, adjust the amount of light emission, and shapes the light beam that is entering the objective. Usually, the higher the magnification used, more light is needed.

Magnification alone is ineffective unless the image seen produces structure and fine detail. The clarity of the image is dependent on the microscope's **resolution**, which is the ability of the lens to distinguish two objects at a particular distance apart. For example, if a microscope has a resolving power of 0.2 µm, the viewer will be able to distinguish two bacteria that are separated by a distance of 0.2 µm or more.

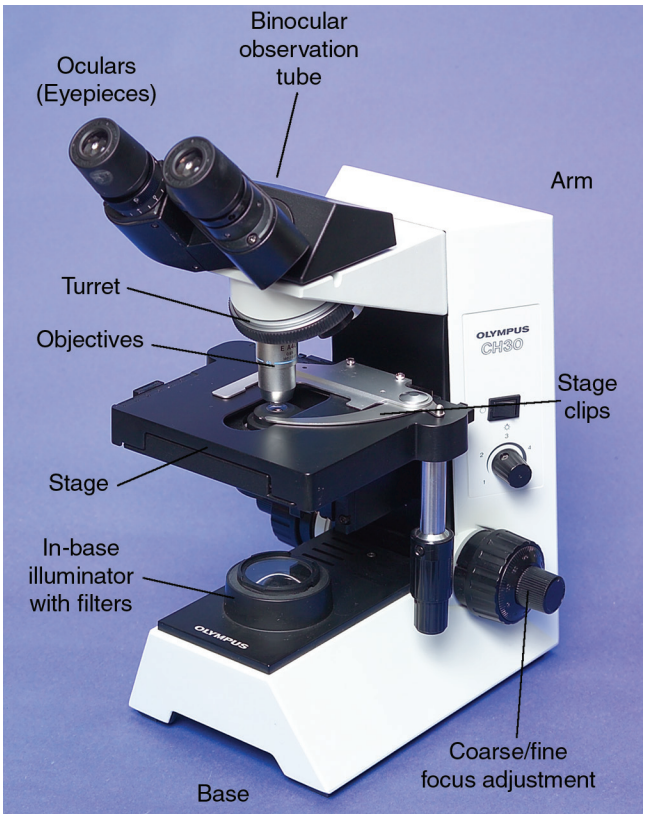


Figure 3-2 Compound light microscope.

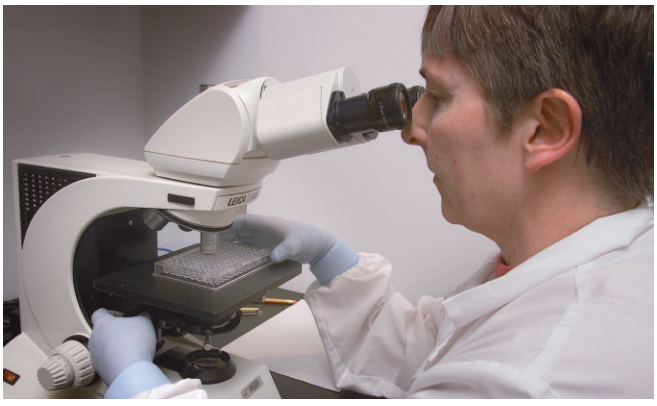
A principle that applies to the use of the compound light microscope is that the shorter the wavelength of light, the greater the resolution. A compound microscope is able to image a bacterium but not a virus. A bacterium is typically 0.5–5.0 µm (500–5,000 nm) and viruses are usually around 0.02 µm (20 nm) in size. The shortest or smallest wavelength of visible light is approximately 0.4 µm (400 nm); therefore the compound microscope is unable to create a clear resolution of the much smaller virus.

Another important principle of microscopy is the **refractive index**. A clear, detailed image of an object under the microscope requires that there be a relatively dramatic or substantial contrast between the specimen being examined and type of medium in which it is suspended. The refractive index of the specimen must be changed to achieve a clear contrast from that of the medium. The refractive index is a measure of the relative velocity at which light passes through a material. The refractive index of microbes is changed through the use of staining procedures, discussed in the next section. Light rays travel in a straight line through a single medium, but staining causes the light rays to pass through a specimen and its medium with different refractive indexes. This causes the light rays to change direction from a straight path to an angle or refractive path at the boundary of the specimen and medium. This refraction of the light increases the contrast between the two. As the light rays travel away from the specimen, they spread out to achieve resolution. The light rays continue to pass through the objective lens, and the image is magnified.

Dark-Field Microscope

The dark-field microscope is actually a bright-field or compound microscope fitted with a dark-field condenser. The dark-field microscope is used when (1) microbes are not visible with the use of the light microscope, (2) the microbes cannot be stained by standard methods, or (3) the staining process distorts the microbes.

The dark-field microscope uses a dark-field condenser that contains an opaque disc in place of the normal condenser. The opaque disc is either inserted below the condenser or is a permanent component of the condenser. The disc blocks light that would enter the objective directly and only permits peripheral rays of light to enter (see Figure 3-3).



Courtesy of CDC/Maryam I. Daneshvar, Ph.D.; Photo by James Gathany

Figure 3-3 CDC researcher reading a microscope agglutination test (MAT) by using dark-field microscopy.

Phase-Contrast Microscope

The phase-contrast microscope is particularly useful because it allows detailed visualization of the internal structures in living microbes. Flagella, granular microbes, and other types of bacteria are examples of organisms that are more effectively examined with the phase-contrast microscope. The degree of detail also eliminates the need to fix or stain the microbes because these are procedures that may alter or kill the microbe.

The microscope is equipped with a special condenser that contains a ring-shaped diaphragm. The light passes through the diaphragm and focuses on the specimen, forming a halo of light around it. The light is also simultaneously focused on the second ring-shaped diffraction plate in the objective lens. The undiffracted and diffracted light rays are then brought into phase or synchronization with each other to produce the image of the specimen.

The principle of phase-contrast microscopes is based on the refractive index. The velocity of light rays is altered by the various internal structures of the microbe as they pass through the specimen. The light rays are bent, or diffracted, and travel in various pathways. This is referred to as being "out of phase," and the differences in phases are seen through the microscope as varying degrees of brightness. The internal structures therefore appear as degrees of brightness against a dark background, allowing an observer to identify the details of the structures.

Fluorescence Microscope

Some microbes are fluorescent, meaning that they absorb ultraviolet light. After absorbing the energy, they emit a longer wavelength of light that is seen with the use of special filters. Some microbes have a natural property of fluorescence and others can be stained with one of a group of dyes called fluorochromes. Microbes stained with a fluorochrome appear as a bright object against a dark field when an ultraviolet light source is used (see Figure 3-4).

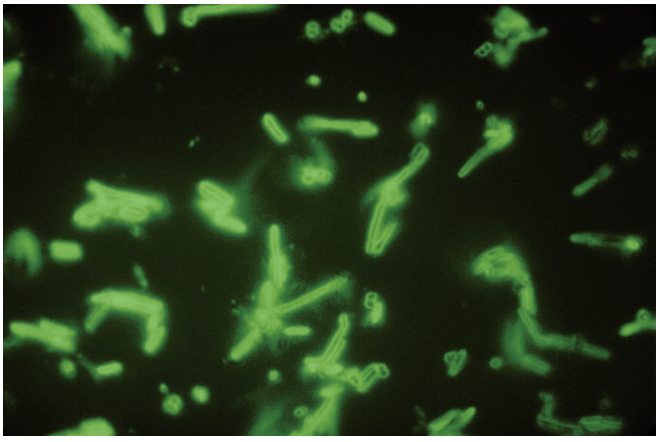
One of the primary uses of fluorescence microscopy is a diagnostic technique called **immunofluorescence**. This technique is useful in detecting bacteria and other pathogens within cells and tissues. It is frequently used in diagnosing the pathogens that cause rabies and syphilis. The following steps are examples of how the immunofluorescence technique is performed:

1. An animal is injected with an antigen, such as a specific type of bacteria.
2. The animal's immune system begins producing specific antibodies against that antigen.
3. The antibodies are removed in a laboratory setting from the serum of the animal.
4. A fluorochrome dye is added and chemically combines with the antibodies.
5. The fluorescent antibodies are then placed on a microscope slide that contains unknown bacteria.
6. If the unknown bacteria are of the same type as those originally injected into the animal, the fluorescent antibodies will bind to the antigens on the surface of the bacteria, causing it to fluoresce.

Fluorochromes have an attraction to specific microbes. Two common dyes are fluorescein and rhodamine. Fluorescein produces a yellow-green fluorescence and rhodamine produces a reddish orange color. The fluorochrome Auramine O is highly absorbed by *Mycobacterium tuberculosis*, causing it to glow yellow when exposed to ultraviolet light. Consequently, the bacteria are visualized as a bright yellow organism against a dark background. *Bacillus anthracis* appears apple green against a dark background when the fluorochrome fluorescein isothiocyanate is absorbed by the bacterium's cell wall.

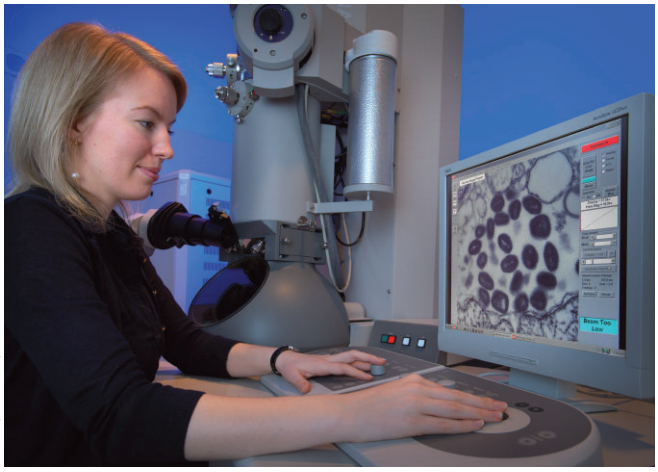
Electron Microscope

The invention of the electron microscope created the ability to visualize viruses, the internal structures of cells, and other objects smaller than 0.2 µm. The electron microscope uses a beam of electrons instead of light; therefore, the resolving power of the electron microscope is much higher than it is for other types of microscopes (see Figure 3-5). The improved resolution is due to the much shorter wavelength of the electrons.



Courtesy of CDC.

Figure 3-4 Microorganisms histochemically processed using the fluorescent antibody (FA) staining method. Visible are both rod-shaped bacilli and small round cocci bacteria.



Courtesy of CDC/Cynthia Goldsmith.

Figure 3-5 CDC intern using a transmission electron (TEM) microscope.

The electron microscope uses an electromagnetic lens instead of a glass lens. The lens focuses a beam of electrons that is traveling through a tube onto the specimen. There are two types of electron microscopes: transmission electron microscope (TEM) and scanning electron microscope (SEM).

Transmission Electron Microscope

The TEM utilizes a focused beam of electrons that are emitted from an electron gun and pass through a very thin section of the specimen. The electron beam is focused on a small portion of the specimen by an electromagnetic condenser lens to direct the beam in a straight line and illuminate the specimen.

The electromagnetic lenses control illumination, magnification, and focus. The specimen is placed on a mesh grid made of copper. The electron beam passes through the specimen and continues by traveling through the electromagnetic objective lens, which is responsible for magnifying the image. Finally, an electromagnetic projector lens focuses the electrons onto a fluorescent screen or photographic plate.

The TEM can resolve objects as close in proximity as 2.5 nm and can be magnified 10,000× to 100,000×. The contrast between specimen and medium is weak, however, because the specimens are extremely thin. This is resolved by the use of a stain that absorbs electrons to produce a darker image. Commonly used stains are lead, tungsten, and uranium. Two methods of staining are used: positive staining, in which the metal is directly fixed to the specimen, and negative staining, which increases the electron opacity of the surrounding background field. Negative staining is most frequently used for the study of the smallest of microbes, such as viral particles.

The resolution of a TEM is very high, providing the ability to study the various layers of a specimen. But a TEM does have the following drawbacks:

1. Electrons have a limited penetrating capability, requiring an ultrathin section of the specimen to be obtained for study. A three-dimensional view of the specimen cannot be obtained due to this limitation.
2. Specimens must be dehydrated, fixed, and viewed under a high vacuum. The treatments do not allow for live microbes to be viewed; in addition, the treatments can cause some distortion of the microbe.

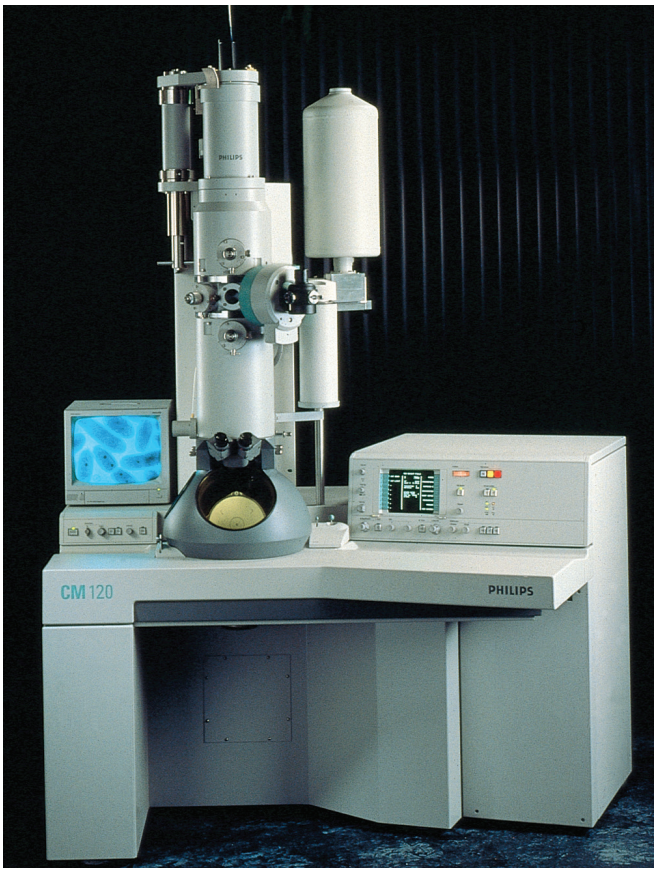
Scanning Electron Microscope

The SEM offers two key advantages over the TEM:

1. A section of specimen does not have to be thinly sliced and prepared for viewing.
2. Three-dimensional (3-D) views are obtained.

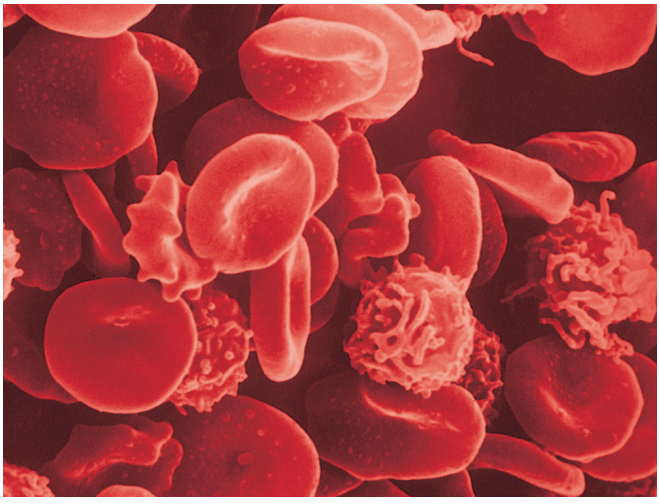
With the SEM, an electron gun produces the beam of electrons called the primary electron beam. The electrons pass through electromagnetic lenses and travel over the surface of the specimen. The primary beam kicks electrons out of the surface of the specimen. These secondary electrons are transmitted to an electron collector, amplified, and used to view the image (see Figure 3-6).

The SEM is particularly useful for viewing the surface structures of whole cells and viruses. It can resolve objects as close together as 20 nm and specimens are magnified 1,000× to 10,000×.



Courtesy of Philips Electronic Instruments Company.

(A)



Courtesy of Philips Electronic Instruments Company.

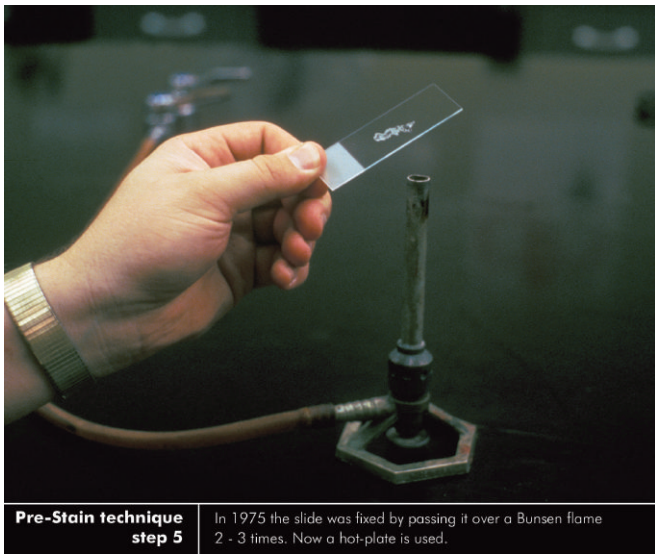
(B)

Figure 3-6 (A) Transmission electron microscope and (B) scanning electron microscope image.

Laboratory Staining Methods

Many microorganisms appear colorless and are therefore difficult to see when viewed through a compound light microscope. Staining is one method of preparing specimens for microscopic examination. Before the staining procedure is performed, the specimen must be "fixed" to the microscope slide or the stain will wash off the microbes (see Figure 3-7).

One method of microbial fixing involves the use of a flamed loop to spread small bacterial samples suspended in broth or water onto a slide. This is called a smear and it is allowed to air dry. Next, the slide is passed over the flames of a Bunsen burner several times, smear side up. The flaming kills the bacteria and fixes or adheres them to the slide. A second method involves all the steps described except that 100 percent methanol is used to fix the smear (see Figure 3-8).



Courtesy of CDC/Dr. Michael Rein.

Figure 3-8 Bunsen burner used in a laboratory to fix a slide preparation.

Simple Stains

A **simple stain** is sufficient for determining the shape of bacteria and viewing basic structures. The method involves the use of a single dye that is applied to the fixed smear, rinsed with water, and dried. A drop of immersion oil is placed directly on the smear to facilitate specimen viewing. Examples of common simple stains are methylene blue, carbolfuchsin, safranin, and crystal violet.

A chemical additive called **mordant**, may be added to the stain to increase the function of the stain. The mordant increases the affinity of a stain for a particular specimen and its ability to coat a cell structure, such as the flagellum, making it thicker and easier to see after the cell is stained with the dye (see Figure 3-9).

Differential Stains

Differential stains vary in their reactions to select types of bacteria, making it easier to distinguish them from other groups. The two most frequently used differential stains are the Gram stain and acid-fast stain.

Gram Stain

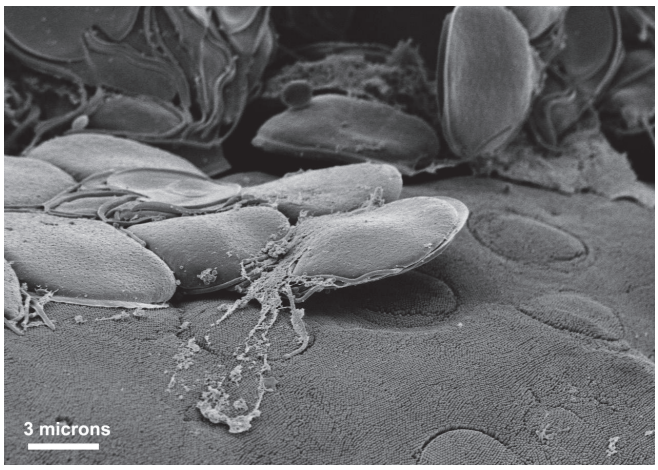
In 1884, the Danish bacteriologist Hans Christian Gram developed the staining method called the Gram stain. The method differentiates bacteria into one of two groups: Gram-positive or Gram-negative. The color of the bacteria at the end of the staining procedure, either purple or red, depends on the chemical composition and thickness of the cell wall of the bacteria (see Figure 3-10). On occasion, a bacterial specimen may display unique characteristics and be categorized into a third type referred to as Gram-variable.

The steps of the Gram stain procedure are as follows:

1. An air-dried, heat-fixed smear is thoroughly covered with the purple dye called crystal violet. The stain is absorbed by all cells and is referred to as a primary stain.
2. After 30 seconds the stain is rinsed off with water and the smear is covered with iodine, a mordant.

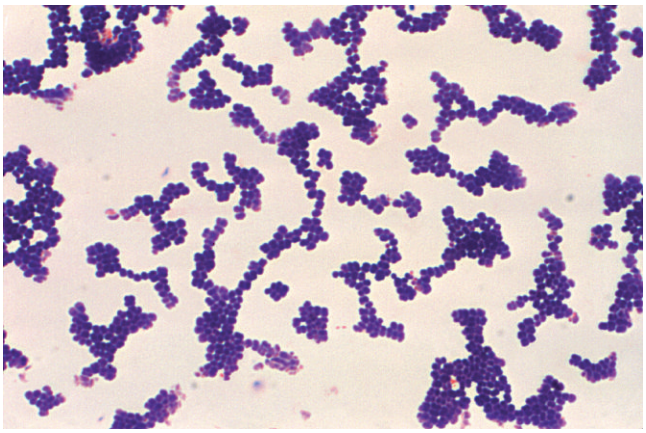
Courtesy of CDC/Dr. Mae Melvin.

Figure 3-7 Trichrome staining materials used in a laboratory setting.



Courtesy of CDC/Dr. Stan Erlandsen.

Figure 3-9 Scanning electron micrograph (SEM) of *Giardia* trophozoites clustered on the intestinal mucosal surface. The *Giardia* species are free-swimming by use of flagella.

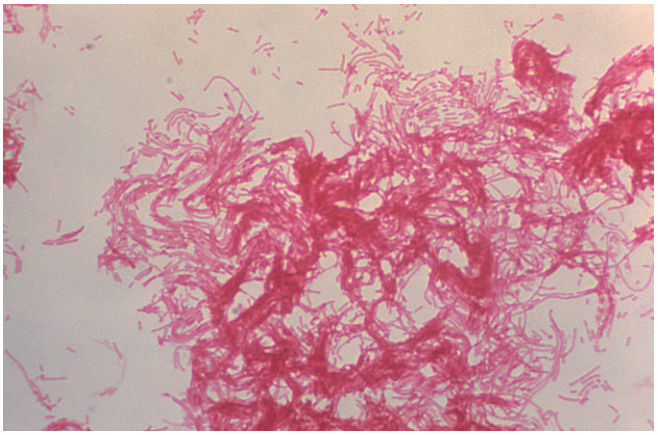


Courtesy of CDC/ Dr. Richard Facklam.

Figure 3-10 Magnified 320×, this photomicrograph revealed the presence of many Gram-positive *Micrococcus mucilaginosis* bacteria. Note that micrographically, these bacteria closely resemble staphylococcal organisms.

3. After 30 seconds the iodine is gently rinsed off with water. Gram-positive and Gram-negative bacteria both appear dark purple.
4. The slide is then coated with the decolorizing agent called ethanol, an organic compound also known as ethyl alcohol. The ethanol removes the purple stain from some bacteria and not from others.
5. The ethanol is rinsed off.
6. The slide is next stained with a basic red dye called safranin. The safranin is termed as a counterstain because it has color contrasting to the primary stain. The stain is left on the slide for 1 minute.
7. The slide is finally rinsed with water, blotted dry, and examined under immersion oil.

Gram-positive bacteria remain purple (or dark blue) from uptake of the crystal violet. The purple color is removed by the ethyl alcohol in Gram-negative bacteria, and they appear red or pink from the safranin counterstain (see Figure 3-11). As previously mentioned, some types of bacteria do not consistently stain purple or red and are referred to as Gram-variable bacteria. Examples of Gram-variable bacteria include *Mycobacterium tuberculosis*, *Clostridium tetani*, and *Yersinia pestis*.



Courtesy of CDC/ Dr. Mike Miller.

Figure 3-11 Under magnification of 1,200×, this Gram-stained photomicrograph revealed the presence of numerous Gram-negative *Haemophilus ducreyi* bacteria.

Acid-Fast Stain

The **acid-fast stain** binds only to bacteria that have a waxy chemical material in their cell wall. The stain is used to identify all the bacteria that are classified in the genus *Mycobacterium* (see Figure 3-12).

The following steps state the procedure for applying the stain:

1. The bright red dye carbolfuchsin is applied to a fixed smear. The microscope slide is heated for several minutes. The heat aids the stain in penetrating the cell wall.
2. The slide is allowed to cool and is rinsed with water.
3. A mixture of acid and alcohol, which acts as a decolorizer, is used to treat the slide. Bacteria (such as the mycobacteria) that retain the red color are said to be acid-fast. The other bacteria that do not retain the stain and appear colorless are non-acid-fast.
4. Methylene blue can be used as a counterstain to stain the non-acid-fast bacteria.

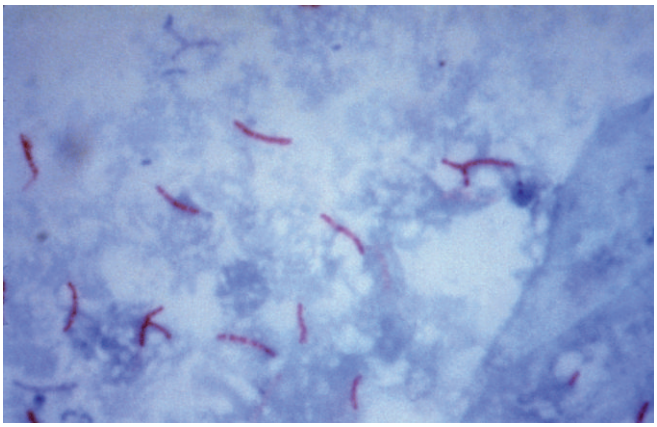
The acid-fast mycobacteria are visualized as red organisms against a blue background in a sputum specimen obtained from a patient positive for tuberculosis.

Special Stains

Special stains are used to stain specific structures of microbes, such as spores or flagella, and aid in identifying the presence of a capsule. The three most common special stains are negative staining for capsules, spore staining, and flagella staining.

Negative Staining for Capsules

A capsule is a gelatinous-like covering that many microorganisms contain. The capsule often protects pathogenic



Courtesy of CDC/ Dr. George P. Kubica.

Figure 3-12 The photomicrograph reveals *Mycobacterium tuberculosis* bacteria using acid-fast Ziehl-Neelsen stain. Magnified 1,000×. The acid-fast stain is retained by *M. tuberculosis*.

microbes from the process known as phagocytosis performed by the protective phagocytes in the host's body. **Virulence**, the degree to which a pathogen can cause disease, can be determined by confirming if a microbe has a capsule.

Capsule staining is difficult because the material that forms the capsule is soluble in water, and rinsing can cause the capsule to be removed. The procedure involves mixing the bacteria in a solution of India ink, a colloidal suspension that provides a dark background for viewing the bacteria. The bacteria can then be stained with a simple stain such as safranin. Because of its chemical composition, the capsule does not absorb the simple stain and a halo appears around the stained bacterial cell.

The India ink is used to demonstrate a negative-staining technique. The stain does not penetrate the capsule, causing the colorless bacteria to appear against a colored background. This provides a sharp contrast between the capsule and the surrounding dark medium.

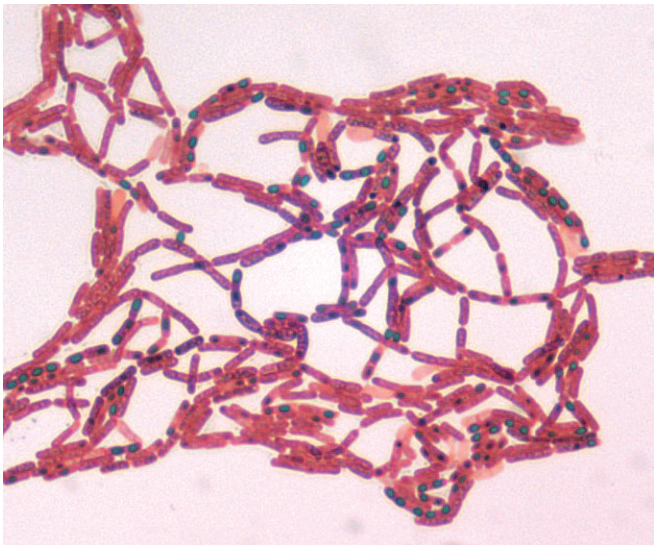
Spore Staining

A select group of bacteria can form a structure called a **spore**. The spore is a dormant structure found within the cell. The cell forms the spore as a way of self-preservation when environmental conditions that allow the cell to live do not exist. The spore is highly resistant to difficult environmental conditions, such as heat, and is very difficult to destroy. When the environmental conditions improve and can support the cell, the spore is released. More discussion on spores is found in later sections.

Spores cannot be stained by methods previously presented because the stains cannot penetrate the wall of the spore (see Figure 3-13).

The following is the procedure used to stain a spore:

1. The primary stain is malachite green. It is applied to a heat-fixed smear and heated approximately 5 minutes until the stain is steaming. The heat aids the stain in penetrating the spore wall.
2. The slide is washed for 30 seconds to remove the malachite green from all the portions of the cell except for the spore.
3. The counterstain safranin is applied to stain the portions of the cell other than the spore.
4. The spore appears green within a red cell.



Courtesy of CDC/ Courtesy of Larry Stauffer, Oregon State Public Health Laboratory.

Figure 3-13 *Bacillus* sp., Malachite Green spore stain, at a 1,000× magnification.

Flagella Staining

The flagella of bacteria, which aid in motility of the cell, cannot be seen through a compound light microscope without staining. **Flagella staining** is a tedious staining procedure that uses a mordant and the stain carbolfuchsin to thicken the flagella. The flagellum can be seen through the light microscope as soon as its diameter is large enough (see Figure 3-14).

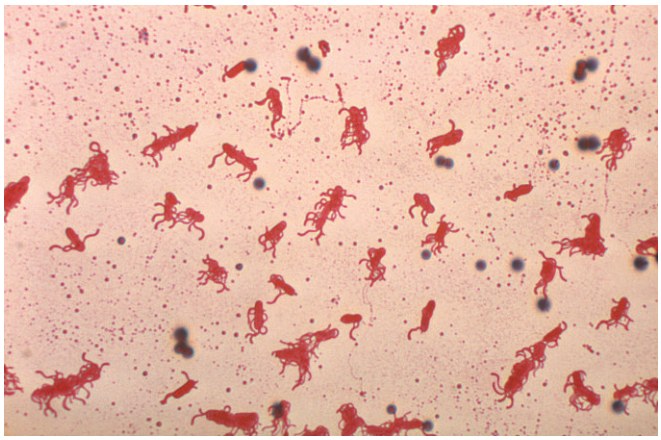
Culture Media

Pure cultures of bacterial species are grown in the laboratory with the use of **culture media**. The culture media (medium is singular) provide a method in which the appropriate nutrients can be delivered to the bacteria in a controlled environment. The microbiologist can easily control the amount of oxygen, heat, and pH available to the bacteria. Optimal conditions for growth are created for the bacteria when combined with the culture medium.

Some types of bacteria can proliferate on almost any type of culture medium, whereas others require special media. The microbial colonies that grow and multiply in or on a culture medium are referred to as the **culture**.

The culture medium must meet the following criteria to be useful:

1. It must contain nutrients, and often species-specific nutrients, to encourage the growth of the microbe that is being cultured.
2. It must contain enough moisture and the proper level of pH.
3. It must be able to be either exposed to or not exposed to oxygen, depending on the requirements of the microbe.



Courtesy of CDC/Dr. William A. Clark.

Figure 3-14 *Bacillus alvei*, Leifson flagella stain.

4. It must be sterile, meaning no other microbes are growing on the medium prior to the addition of the microbes that are being grown.
5. It must be able to withstand the temperature conditions created during incubation.

Growth media can be used in broth (liquid) form, which is available in tubes, or in a solidified form, created by the addition of **agar** that is poured into Petri dishes. The bacteria can then be grown on the solid surface of the agar medium. Agar is a complex polysaccharide and has a long history of use as a thickener in foods such as jellies, soups, and ice cream.

Robert Koch and his associate Walter Hesse are credited with the discovery of agar. The actual credit, however, belongs to Hesse's wife, who knew that her husband and Koch were struggling to find an improved method of growing pathogens without the use of gelatin. She told Walter about a substance her grandmother used in the tropics to make jams and jellies remain solid in the hot, humid tropical temperature. The substance is agar, obtained from red marine algae growing along the coasts of southern California, China, Japan, and Malaysia.

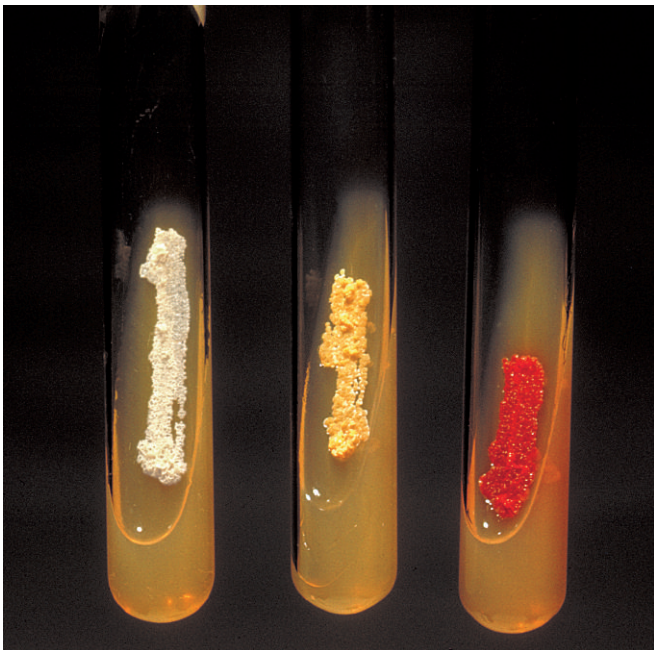
Agar is a solidifying agent that is added to the medium. It has valuable properties that make it irreplaceable in the laboratory and no other satisfactory substitute has ever been found. Agar remains solid because few microbes have the ability to break it down. Agar melts at the boiling point of water and then solidifies at approximately 40°C. In the laboratory, agar is maintained at approximately 50°C and it does not injure the bacteria. The solidified agar can be incubated up to 100°C before it liquefies. This is especially useful when thermophilic, or heat-preferring bacteria are to be grown.

Agar media are contained not only in Petri dishes but also in test tubes. The test tubes are called **slants** because the agar is allowed to solidify with the tube positioned at an angle. This slanted agar surface provides the bacteria with a larger surface area for growth. Agar solidified in a tube in a straight, vertical fashion is referred to as a "deep" (see Figure 3-15).

There are three categories of media: enriched, selective, and differential. There are different types of agar within each category that serve particular uses in the laboratory. The various categories of media are not mutually exclusive. Some of the media fall into two or more categories according to their action.

Enriched Media

Enriched media can be either a solid or a broth that contains a supply of nutrients that promote the growth of fastidious organisms. Bacteria that are recognized as having complex nutritional requirements are referred to as **fastidious**. They will not grow outside of living cells and must be cultured in living animals, cell cultures, or chicken egg embryos.



Courtesy of CDC/ Dr. David Berd.

Figure 3-15 Slant cultures demonstrating variations in colonial appearance among aerobic *Actinomycetes* spp. White indicates *A. madurae*; yellow indicates *Nocardia asteroides*; and red indicates *Micromonospora* sp.

Blood agar and **chocolate agar** are two popular types of solid enriched media. Blood agar is a combination of nutrient agar and sheep erythrocytes, otherwise known as red blood cells. It is used to cultivate certain microorganisms, including *Staphylococcus epidermidis, Diplococcus pneumoniae,* and *Clostridium perfringens*.

Chocolate agar, which is both an enriched and a selective medium, is a combination of nutrient agar and powdered hemoglobin. The name is given only due to the brown color of the agar. Microorganisms commonly cultivated with the use of chocolate agar include *Haemophilus influenzae, Neisseria gonorrhoeae,* and *Neisseria meningitidis*.

Selective Media

Selective media have chemical inhibitors that prevent the growth of particular species of microbes while allowing the growth of the desired species. **MacConkey agar**, which is both a selective and differential medium, is a combination of bile salts, lactose, and crystal violet. Gram-negative bacteria are able to ferment lactose and produce pink colonies, distinguishing them from the colorless bacteria that cannot ferment the lactose. MacConkey agar specifically differentiates between lactose-fermenting (LF) and non-lactose-fermenting (NLF) bacteria. One important use of the agar is distinguishing between the pathogenic *Salmonella* bacteria and other types that are related.

Other frequently used types of selective media include the following:

1. Phenylethyl alcohol (PEA) agar: A blood agar to which inhibitory substances have been added. Selective for Gram-positive bacteria.

2. Thayer-Martin agar: A type of chocolate agar that contains extra nutrients and an antimicrobial agent. It is selective for *N. gonorrhoeae.*
3. Mannitol salt agar (MSA): Only allows salt-tolerant bacteria to grow.
4. Colistin-nalidixic acid (CNA): Also a blood agar to which inhibitory substances have been added. Selective for Gram-positive bacteria.
5. Eosin methylene blue (EMB): A differential and selective medium. It contains substances that indicate fermentation of sugars and the resulting change in pH. It also contains crystal violet to inhibit the growth of Gram-positive microorganisms.

Differential Media

Differential media can make it easier to distinguish specific colonies of bacteria from other colonies that are growing on the same Petri dish. Blood agar, which is also an enriched medium, is frequently used to identify bacteria that destroy erythrocytes. For example, *Streptococcus pyogenes*, the bacteria responsible for causing strep throat, display a clear ring around the colony where the bacteria have lysed the surrounding erythrocytes.

MSA is used to identify *Staphylococcus aureus*, which grows readily on MSA and also ferments mannitol. Fermentation turns the MSA medium to a yellow color.

Reducing Media

The cultivation of anaerobic bacteria is difficult for the microbiologist. Special media called **reducing media** must be used to prevent the destruction of the bacteria by oxygen. The reducing medium contains chemicals, such as sodium thioglycolate, that combine with oxygen to eliminate it from the environment. To grow pure cultures of anaerobes, the reducing medium must be stored in tightly capped test tubes. The medium is then heated immediately before use so that the absorbed oxygen is eliminated.

When the culture must be grown in a Petri dish so that individual colonies can be observed, special jars are used that can contain several Petri dishes in an oxygen-free environment.

The oxygen is removed through the following process:

1. A chemical combination of sodium bicarbonate and sodium borohydride is placed in the jar, and moistened with just a few milliliters of water. The jar is tightly sealed.
2. The chemical reaction with the water produces hydrogen and carbon dioxide.
3. Next, the hydrogen and oxygen combine together in a chemical reaction and forms water.
4. The result is the disappearance of oxygen in a short period of time.

The carbon dioxide that is produced aids the growth of many types of anaerobic bacteria.

Microbiologists who work with anaerobes on a daily basis use an **anaerobic chamber**. The chamber is equipped with air locks and filled with inert gases. Researchers are able to handle and manipulate the equipment by inserting their hands into airtight gloves that are fixed to the wall of the chamber.

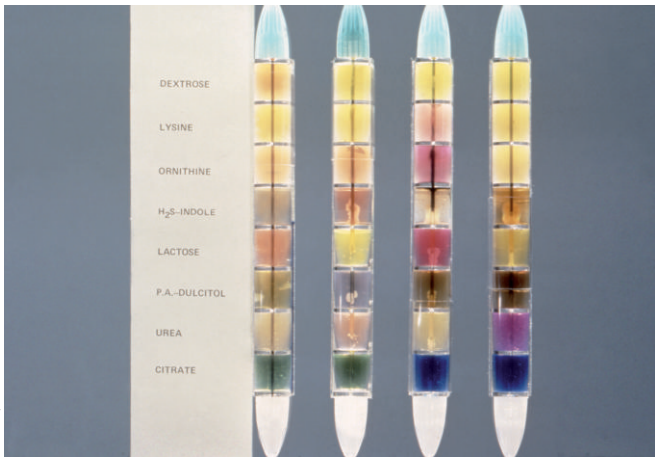
Rapid Identification Testing

A number of manufacturers have developed kits for use by healthcare providers to allow for expedited test results for certain types of pathogens. In response to the HIV/AIDS epidemic, screening efforts have been greatly expanded over time. One of the ways in which to encourage testing by individuals was to make access and results easy and discreet. Physicians in pediatric offices utilize rapid result tests for Streptococcal infections to begin immediate pharmacological treatment rather than having to wait for 24- to 48-hour culture results.

In the laboratory setting, manufactured kits have also been developed that incorporate multiple types of culture media in one device for inoculation. The Enterotube™ II Prepared Media Tube was developed by Becton Dickinson (BD) (see Figure 3-16). Another test kit designed for detection of anaerobic Gram-negative bacilli is the Rapid ID 32A tray by bioMérieux (see Figure 3-17).

Serology

When a microorganism enters an animal's body, it stimulates the host's immune system to produce **antibodies**. Antibodies are proteins located in the circulatory system that bind with the specific bacterium or antigen that was the cause of their production. **Antisera** (singular, antiserum) are solutions of



Courtesy of CDC/Dr. Theo Hawkins.

Figure 3-16 The Enterotube® identification kit is a multi-test system designed to confirm the identification of different isolates. Each tube contains eight different agar preparations, allowing a diagnostician to perform a number of simultaneous biochemical tests.

Courtesy of CDC/ Melissa Dankel; Photo by James Gathany.

Figure 3-17 Centers for Disease Control (CDC) laboratorian holding a multi-well Rapid ID 32A tray (biomérieux, Inc.) used for the identification of anaerobic Gram-negative bacilli.

antibodies that are commercially produced and used to identify microorganisms. An unknown bacterium that is isolated from a patient can be tested against the antisera and identification can be achieved. Two popular tests using antisera are the **enzyme-linked immunosorbent assays (ELISAs)** and slide agglutination test.

ELISA is used extensively in the laboratory because test results can be quickly obtained and the results can be analyzed by a computer scanner. The test involves placing known antibodies in the depressions or wells of a microplate. The unknown bacteria are placed in each well. A reaction between the bacteria and antibodies identifies the type of bacteria. ELISA is commonly used in the diagnosis of HIV (see Figure 3-18).

The slide agglutination test involves placing samples of an unknown bacterium in a drop of saline on several microscope slides. Next, a different known antiserum is placed in each sample. If the bacteria agglutinate (clump together), a positive test result is achieved. The agglutination is caused by the combination of the bacteria that have an affinity for the antiserum that was added.

Specialized Laboratory Analyses

Numerous other forms of laboratory analyses are performed to identify microorganisms. Routinely, computers are tasked

Courtesy of CDC/ Hsi Liu, Ph.D., MBA, James Gathany.

Figure 3-18 CDC microbiologist using an enzyme-linked immunosorbent assay (ELISA) test to develop a method for the rapid detection of HIV p24 antigen in blood samples.

with compiling the complex results and creating comprehensive reports for scientists and physicians to develop their research protocols or treatment plans. Some of the specialized testing methods are included in this section.

Catalase Test

Catalase is an **enzyme** produced by many living cells, including a number of species of bacteria. Hydrogen peroxide is a by-product of some normal cellular metabolic processes. The enzyme allows for the rapid breaking down of hydrogen peroxide (H_2O_2), a toxic chemical to cells, into harmless molecules of water (H_2O) and oxygen (O_2). Many organisms survive, in part by having defense mechanisms that allow them to escape or repair the oxidative damage caused by hydrogen peroxide. Some species of bacteria are able to perform cellular detoxification by producing catalase and are classified as being either catalase-positive or catalase-negative. The presence or absence of catalase in bacterial cultures provides an easy identification marker for clinicians.

The laboratory test involves placement of a carefully obtained sample from a culture onto a microscope slide placed into a Petri dish (optional but recommended). The technician takes care not to inadvertently pick up culture media, especially if using a blood agar, which might create a false-positive result. A single drop of 3 percent hydrogen peroxide is placed onto the bacterial sample and the cover of the Petri dish is positioned to prevent catalase aerosols. If effervescence (bubbling) is seen, then the test result is positive. In some weak responses, it may be necessary to examine the slide under the microscope. The test can also be performed using test tubes. *N. gonorrhoeae* produces vigorous bubbling reactions. *S. aureus* produces positive catalase reactions, and *Staphylococcus pyogenes* exhibits a negative reaction. *Streptococcus* and *Enterococcus* species usually are catalase-negative.

Coagulase Test

Coagulase is another enzyme produced by some bacterial species that coagulates (clots) blood plasma. The coagulase test is a laboratory study performed on Gram-positive, catalase-positive species of bacteria to definitively identify the coagulase-positive *S. aureus* species. Coagulase is considered a virulence factor of *S. aureus* because formation of a clot around the site of infection caused by these bacteria likely protects it from the body's natural inflammatory process of phagocytosis.

Two versions of the coagulase test are also used for definitive identification of *Staphylococcus* species. A laboratory slide preparation is inoculated with the bacterial specimen and a specific type of prepared plasma. In a matter of seconds, the positive reaction is observed as a clumping together of the bacterial cells. This provides a presumptive reading of *S. aureus*, due to the property of positive coagulase reaction. If, however, the slide test is negative, then further testing by the tube method is performed. The bacteria is introduced into prepared plasma in a test tube and allowed 24 hours to develop. At that time if there remains no reaction, the organism is deemed coagulase-negative and, if other studies agree, the species is determined to most likely be *S. epidimidis* or another coagulase-negative *Staphylococcus* (CoNS) and *S. aureus* is ruled out.

Amino Acid Sequencing

DNA is responsible for the encoding of the proteins that contain the base sequence of amino acids. Amino acid sequencing is based on the evolutionary course of two organisms. The changes in the DNA sequence that encodes the proteins in the two organisms will be either similar or dissimilar depending on the length of time that has taken place between the evolution of two microbes. The similarity of the DNA sequence can aid in determining the evolutionary closeness of the microbes by comparing the amino acid sequences from proteins of two different microbes. The more similar the proteins, the more related the microbes are.

Phage Typing

Phage typing, like serologic testing, is useful in determining the origin and course of a disease, such as healthcare-associated infections (HAIs). Phage typing indicates to which phages a bacterium is susceptible. Bacteriophages are bacterial viruses that are responsible for the lysis of bacteria they invade. The phages infect only specific types of bacteria in a particular species. Phage typing is used most often to identify strains of *S. aureus*, *Vibrio cholerae*, *Salmonella typhi*, and *Pseudomonas aeruginosa*.

To perform phage typing, a Petri dish is covered with bacteria on the agar growth medium. Drops of different phages are placed on the bacteria. Clear spaces called **plaques** subsequently appear on the dish. These areas indicate where the phages have lysed the bacteria. Phage typing is important in establishing the source of a surgical wound infection that may have been caused by an individual on the surgical team. The bacteria that are isolated from the surgical wound can be shown to have the same sensitivity to phages as those bacteria isolated from a member of the surgical team. This establishes the source carrier, whether the surgeon, nurse, surgical assistant, or surgical technologist as the source of infection or clears them.

As an example, if a staphylococcal infection appears to be associated with the surgery department, then all surgery personnel could be cultured for *S. aureus*. Positive cultures would be phage-typed and compared with the phage type of the cultures from the infected surgical patients. This would identify the colonized individual responsible for the spread of the infection and allow them to seek treatment for what may have been an unrecognized, asymptomatic infection.

Flow Cytometry

Flow cytometry is unique in that a culture of bacteria is not needed to identify the type. The process involves the flow of fluid through a small opening at the bottom of the tube. Bacteria are detected by the difference in electrical conductivity between the bacterial cells and the fluid. If a laser is used to illuminate the fluid as it travels through the opening, then the reflection or scattering of the light provides information

pertaining to the size of the cell, its density, and morphology. The information is analyzed by a computer.

Nucleic Acid Hybridization

The double strands of DNA are held together by hydrogen bonds. If the strands are exposed to heat, then the bonds break and the two strands separate. When the single strands are cooled, they rejoin to form a double strand similar to the original. This technique is used on separate DNA strands from two different microorganisms to determine the similarity between the DNA base sequences of the two microbes.

The assumption of the test is that if two species are similar, their nucleic acid sequence will also be similar. The test reveals the extent of the DNA strands' ability from one microorganism to hybridize (bind) with the DNA strands of the other microorganism. The stronger the degree of **hybridization** is, the greater the similarity.

Another method of hybridization involves RNA. RNA is a single strand and is transcribed from one of the double strands of the DNA. Therefore, the strand of RNA should hybridize with the separated strand of DNA from which it was transcribed. The DNA–RNA hybridization can then be used to determine the degree of relationship between the two microorganisms in the same manner as the DNA–DNA hybridization.

Nucleic Acid–Base Composition

Another effective method for classifying microbes with evolutionary relationships is the determination of the nitrogenous-base composition of DNA. The base composition is expressed as a percentage of guanine plus cytosine, or G+C. The base composition in a single species is fixed and does not change. Therefore, by comparing the G+C content of different species, the degree of interrelationship can be investigated.

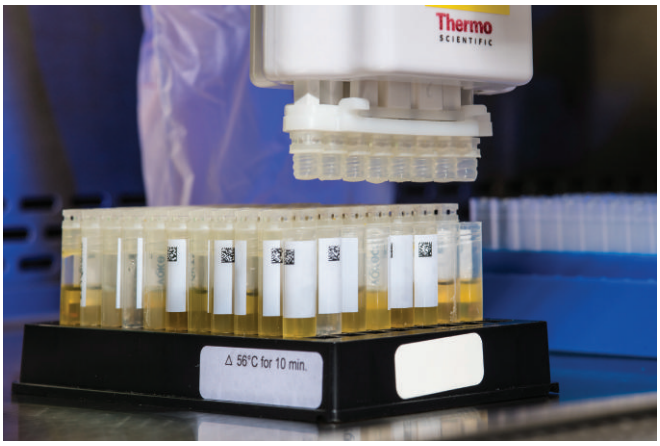
Two microbes that are similar will have many identical genes and similar amounts of the various bases in their DNA. However, if the difference is more than 10 percent in their percentage of G–C pairs, then the two microorganisms are most likely not related. For example, if one bacterium's DNA contains 30 percent G–C and the other has 65 percent G–C, then these two microbes are probably not related.

The base sequences of different microorganisms are compared by using the restriction enzyme test. The DNA from two microorganisms is treated with the same restriction enzyme. The restriction fragments are separated by the process of electrophoresis on a layer of agar. The number and sizes of restriction fragments produced by the different microbes are compared to reveal information about their genetic likeness or difference. The patterns are called DNA fingerprints, and the more similar the DNA fingerprints, the more closely related the microorganisms.

DNA fingerprinting is very important in determining the source of healthcare-associated infections. As an example, a high percentage of patients undergoing coronary artery bypass grafting at a hospital in the Midwest were developing postoperative infections caused by *Rhodococcus bronchialis*. The DNA fingerprints of the patients' bacteria and the bacteria of a nurse were discovered to be identical. The infections were stopped by identifying the infected nurse and making sure that proper aseptic technique was practiced.

The real-time polymerase chain reaction (PCR) test, also known as a quantitative polymerase chain reaction (qPCR), works to amplify, as well as quantify, a specific or targeted DNA molecule. The detection of the DNA molecule in question occurs in real-time, rather than having to wait until the end of the conventional test. In critical cases of drug-resistant strains of pathogens, this allows therapeutic measures to be prescribed more quickly and efficiently.

The SARS-CoV-2 novel coronavirus that causes the COVID-19 infection is widely believed to have been first recognized in the latter months of 2019 in Wuhan, a city in the Hubei Province in China and subsequently became a worldwide pandemic. The incredible speed of global transmission required researchers to develop laboratory test methods that should be rapid and accurate and could be performed in a multitude of conditions throughout the world. The nucleic acid amplification tests (NAATs) and antigen tests were used as diagnostic tests to detect SARS-CoV-2 infections. The requirement of 1–3 days for laboratory NAATs was a barrier to quick diagnosis and recommendations for immediate isolation or quarantine of infected individuals to reduce transmission. Quicker, less expensive, and relatively equally accurate, point-of-care antigen tests were developed with results available in about 15–45 minutes. Current diagnostic tests for coronavirus, based on its genomic characteristics include reverse transcriptase polymerase chain reaction (RT-PCR), real-time reverse transcription PCR (rRT-PCR), reverse transcription loop-mediated isothermal amplification (RT-LAMP), and real-time RT-LAMP. The RT-LAMP tests rely on slightly heating cells in the specimen sample, causing cell lysis and nucleic acid release, which can be used as template for the visible color change indicating amplification of nucleic acids. The diagnostic test gives results in approximately 30 minutes (see Figure 3-19).



James Gathany/CDC

Figure 3-19 Samples for SARS-CoV-2 antibody testing. Serological testing is used to detect antibodies, which indicate past infection with the virus that causes COVID-19 and is important to the understanding of disease prevalence within a population.

Alternative Biosensor Technology

A diagnostic challenge for healthcare providers treating patients in a pandemic, such as COVID-19, has been that the clinical manifestations of COVID-19 are often non-specific. Biosensor technology, including Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), CRISPR-Cas9 or the newer CRISPR-Cas-13 DNA and RNA genome editing, and diagnostic tools are being explored as potential alternatives in traditional diagnosis and therapeutic approaches.

Another technology designed to quickly detect specific viral pathogens such as SARS, COVID, and MERS utilizes a binding protein that attaches to coronavirus fragments. The resulting protein bound viral nanobody is treated with a series of biochemical linkers that adhere it to a thin layer of gold which acts as a semiconductor. An electrical charge is applied to the plate and a device known as an organic electrochemical transistor measures the levels of and changes in current flow in response to the presence of the viral nanobody-bound particles. Researchers are seeking to develop user-friendly devices that are capable of rapid, inexpensive, and accurate diagnostic results in anticipation of potential future viral global pandemics.

Hanging Drop Technique

Bacterial motility (ability to move) can be assessed using the hanging drop technique of observing live bacteria. Robert Koch first utilized this method of studying bacteria in the late 1800s. A special laboratory slide with a concave depression in the center is used along with a coverslip prepared with petrolatum around the edges to keep it securely attached to the slide. A sterilized loop is used to transfer a drop of fluid to be studied onto the coverslip. The slide with the depression is placed over the cover slip and then turned over carefully so that the drop remains suspended from the coverslip over the center well of the slide. An advantage of this type of laboratory test is that the specimen does not dry out due to the intensity of the microscope light as quickly as a standard wet mount slide preparation (see Figure 3-20).

Numerical Taxonomy

Numerical taxonomy involves the comparison of morphologic and biochemical characteristics, amino acid sequence, percentage of G–C pairs, and many other characteristics of microbes to aid in determining relationships. A similarity index is calculated with the use of a computer to determine the similarities. Essentially, the computer matches the characteristics of each microorganism against other microorganisms. The greater the number of characteristics shared by two or more organisms, the greater the chance they are related. A match of 90 percent or more of these characteristics usually indicates a single species.

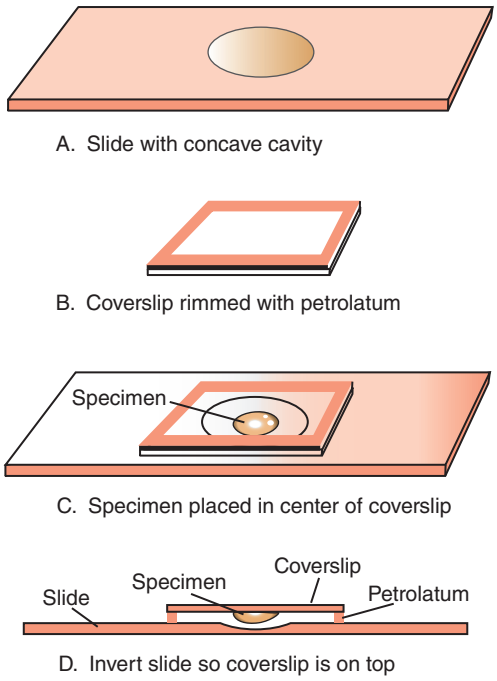


Figure 3-20 Preparation of a hanging drop slide. This technique is used to study living bacteria.

MICRO NOTES

"This Is Just a Test"

The CDC's section on Laboratory Quality Assurance and Standardization Programs states, "More than a billion laboratory tests that identify and measure chemicals, such as lead or cholesterol, are performed each year in the United States. The test results have a significant influence on medical decisions. Given the importance of laboratory test results, the Centers for Disease Control and Prevention's (CDC) National Center for Environmental Health has programs to help assure the quality of these data so patients and healthcare providers (as well as researchers and public health officials) can be confident that laboratory test results they receive are accurate." As members of the surgical team, we understand the importance of accurate microbial testing of surgical specimens as well as in monitoring of the instrument sterilization processes we rely on to prevent SSIs. Evaluation of the testing is an important quality control measure for all involved, from the CDC down to a local hospital laboratory or sterile processing department.