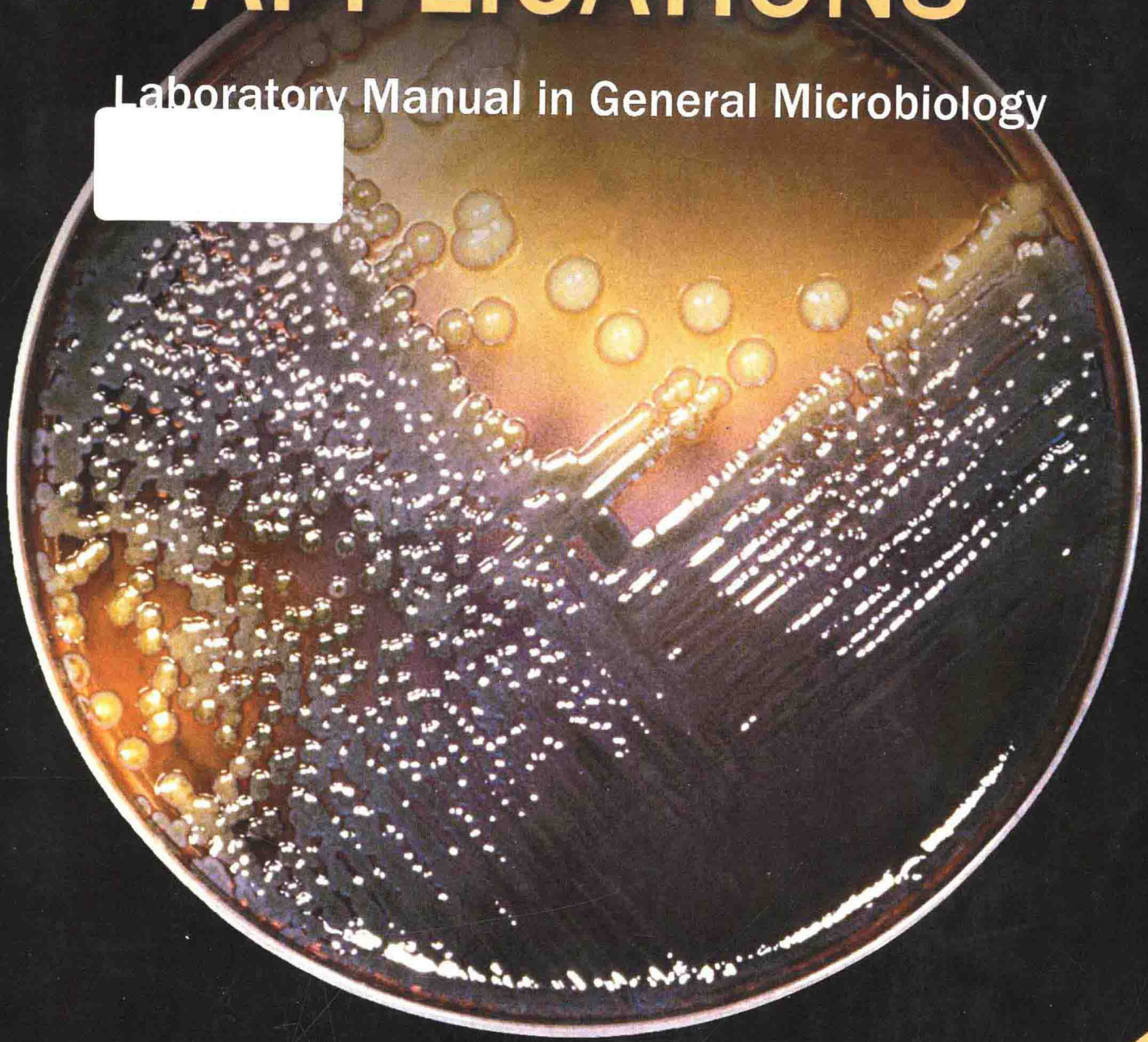
THIRTEENTH EDITION

Benson's MICROBIOLOGICAL APPLICATIONS

Laboratory Manual in General Microbiology



Alfred Brown / Heidi Smith

COMPLETE VERSION

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Benson's Microbiological Applications

Laboratory Manual in General Microbiology

Thirteenth Edition

Alfred Brown
Emeritus Professor, Auburn University

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Mc Graw Hill Education

BENSON'S MICROBIOLOGICAL APPLICATIONS: LABORATORY MANUAL IN GENERAL MICROBIOLOGY, COMPLETE VERSION, THIRTEENTH EDITION

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Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

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About the Authors

Alfred Brown

Emeritus Professor of Microbiology
Auburn University

B.S. Microbiology, California State College, Long Beach

Ph.D. Microbiology, UCLA

Research My research has focused on the physiology of the purple nonsulfur bacteria. This has involved how bacteriochlorophyll and photosynthetic membrane synthesis are coordinated. Herbicides, such as atrazine, have been used to determine the binding site for ubiquinone in photosynthetic electron transport. Binding occurs on the L-subunit, a protein in the photosynthetic reaction center. Resistance to atrazine involves a single amino acid change in the L-subunit that prevents the herbicide from binding to the protein and inhibiting electron transport. This is comparable to how atrazine inhibits electron transport in plants and how resistance to these herbicides develops in weed populations. My laboratory also investigated how the sulfonylurea herbicides inhibit acetolactate synthase, a crucial enzyme in the pathway for branched-chain amino acids. Most recently, I and my graduate students consulted for a company that manufactures roofing shingles. Because of the presence of calcium carbonate in shingles, cyanobacteria can easily grow on their surface, causing problems of contamination. The resulting discoloration caused by these bacteria on shingles has caused significant financial losses to the industry. My laboratory isolated various species of cyanobacteria involved in the problem and taxonomically characterized them. We also tested possible growth inhibitors that might be used in their control.

Teaching Dr. Brown has taught various courses in microbiology over a teaching career that spans more than 30 years. Courses have included general microbiology, medical microbiology, microbial physiology, applied and environmental microbiology, photosynthesis, microbiological methods, and graduate courses, such as biomembranes. In 2008, Dr. Brown retired from the Auburn University faculty as an emeritus professor of microbiology. At present, he continues to work on this manual and travel extensively.

Administration During his tenure at Auburn University, Dr. Brown served as the director of the University Electron Microscope Facility. He also served as the chair of the Department of Botany and Microbiology and the chair of the Department of Biological Sciences.

Heidi Smith

Front Range Community College
B.A. Biology/Pre-Medicine, Taylor University, IN

M.S. Biology, Bowling Green State University, OH

Heidi Smith is the lead faculty member for microbiology at Front Range Community College in Fort Collins, CO, and teaches a variety of biology courses each semester including microbiology, anatomy/physiology, and biotechnology. Heidi has also served as the director of the Honors Program at the college for five years, working with a group of faculty to build the program from the ground up.

Student success is a strategic priority at FRCC and a personal passion of Heidi's, and she continually works to develop professionally in ways that help her do a better job of reaching this important goal. Throughout the past few years, Heidi has had the opportunity to collaborate with faculty all over the country in developing digital tools, such as LearnSmart, LearnSmart Labs, and Connect, to facilitate student learning and measure learning outcomes. This collaborative experience and these tools have revolutionized her approach to teaching and have dramatically affected student performance in her courses, especially microbiology hybrid courses where content is delivered partially online.

Heidi is an active member of the American Society for Microbiology and has presented instructional technology and best online and face-to-face teaching practices on numerous occasions at the annual conference for undergraduate educators. She also served as a member of the ASM Task Force on Curriculum Guidelines for Undergraduate Microbiology Education, assisting in the identification of core microbiology concepts as a guide to undergraduate instruction.

Preface

Benson's Microbiological Applications has been the "gold standard" of microbiology laboratory manuals for over 30 years. This manual has a number of attractive features that resulted in its adoption in universities, colleges, and community colleges for a wide variety of microbiology courses. These features include user-friendly diagrams that students can easily follow, clear instructions, and an excellent array of reliable exercises suitable for beginning or advanced microbiology courses.

In revising the lab manual for the thirteenth edition, we have tried to maintain the proven strengths of the manual and further enhance it. We have updated the introductory material of the fungi, protozoa, and algae to reflect changes in scientific information. Finally, the names of microorganisms used in the manual are consistent with those used by the American Type Culture Collection. This is important for those users who rely on the ATCC for a source of cultures.

Guided Tour Through a Lab Exercise

Learning Outcomes

Each exercise opens with Learning Outcomes, which list what a student should be able to do after completing the exercise.

Learning Outcomes

After completing this exercise, you should be able to

1. Prepare a negative stain of bacterial cells using the slide-spreading or loop-spreading techniques.
2. Use the negative stain to visualize cells from your teeth and mouth.
3. Discern different morphological types of bacterial cells in a negative stain.

Introduction

The introduction describes the subject of the exercise or the ideas that will be investigated. It includes all of the information needed to perform the laboratory exercise.

Bacteriophages are viruses that infect bacterial cells. They were first described by Twort and d'Herelle in 1915 when they both noted that bacterial cultures spontaneously cleared and the bacteria-free liquid that remained could cause new cultures of bacteria to also clear. Because it appeared that the cultures were being "eaten" by some unknown agent, d'Herelle coined the term *bacteriophage*, which means "bacterial eater." Like all viruses, bacteriophages, or phages, for short,

First and Second Periods

In many cases, instructions are presented for two or more class periods so you can proceed through an exercise in an appropriate fashion.

First Period

(Inoculations and Incubation)

Since six microorganisms and three kinds of media are involved in this experiment, it will be necessary for economy of time and materials to have each student work with only three organisms. The materials list for this

Second Period

(Culture Evaluations and Spore Staining)

Remove the lid from the GasPak jar. If vacuum holds the inner lid firmly in place, break the vacuum by sliding the lid to the edge. When transporting the plates and tubes to your desk *take care not to agitate the FTM tubes*. The position of growth in the medium can be easily changed if handled carelessly.

Materials Needed

This section lists the laboratory materials that are required to complete the exercise.

Materials

- microscope slides
- broth cultures of *Staphylococcus*, *Streptococcus*, and *Bacillus*
- Bunsen burner
- wire loop
- marking pen
- slide holder (clothespin)

Procedures

The procedures and methods provide a set of detailed instructions for accomplishing the planned laboratory activities.

Scrub Procedure

The two members of the class who are chosen to perform the surgical scrub will set up their materials near a sink for convenience. As one student performs the scrub, the other will assist in reading the instructions and providing materials as needed. The basic steps,

Illustrations

Illustrations provide visual instructions for performing steps in procedures or are used to identify parts of instruments or specimens.

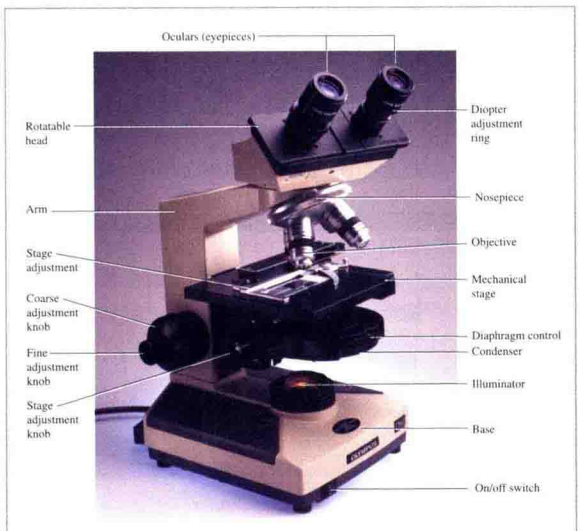


Figure 1.2 **The compound microscope.**
© Charles D. Winters/Science Source.

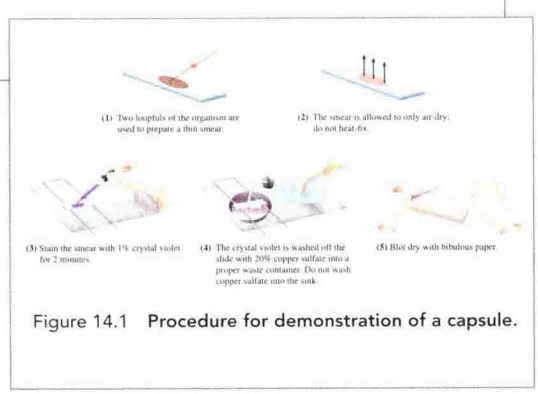
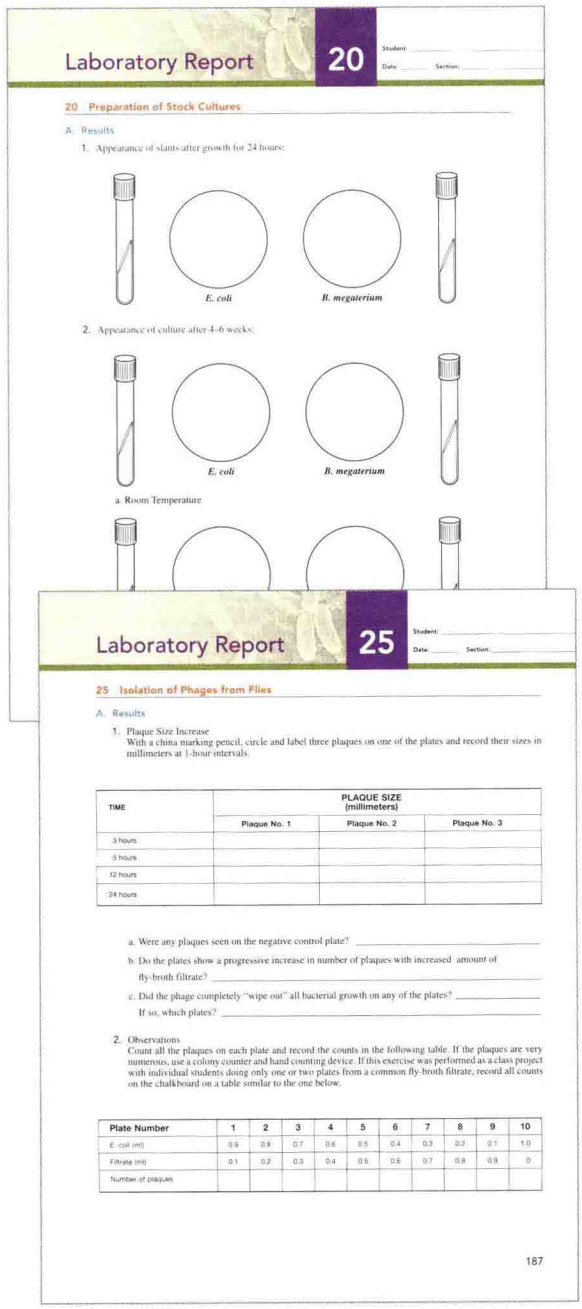


Figure 14.1 **Procedure for demonstration of a capsule.**



Laboratory Report 20

Student: _____
Date: _____ Section: _____

20 Preparation of Stock Cultures

A. Results

1. Appearance of slants after growth for 24 hours:

E. coli *B. megaterium*

2. Appearance of culture after 4–6 weeks:

E. coli *B. megaterium*

a. Room Temperature

Laboratory Report 25

Student: _____
Date: _____ Section: _____

25 Isolation of Phages from Flies

A. Results

1. Plaque Size Increase
With a china marking pencil, circle and label three plaques on one of the plates and record their sizes in millimeters at 1-hour intervals.

TIME	PLAQUE SIZE (millimeters)		
	Plaque No. 1	Plaque No. 2	Plaque No. 3
3 hours			
5 hours			
12 hours			
24 hours			

a. Were any plaques seen on the negative control plate? _____

b. Do the plates show a progressive increase in number of plaques with increased amount of fly-broth filtrate? _____

c. Did the phage completely "wipe out" all bacterial growth on any of the plates? _____
If so, which plates? _____

2. Observations
Count all the plaques on each plate and record the counts in the following table. If the plaques are very numerous, use a colony counter and hand counting device. If this exercise was performed as a class project with individual students doing only one or two plates from a common fly-broth filtrate, record all counts on the chalkboard on a table similar to the one below.

Plate Number	1	2	3	4	5	6	7	8	9	10
E. coli (ml)	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	1.0
Filtrate (ml)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0
Number of plaques										

187

Laboratory Reports

A Laboratory Report to be completed by students immediately follows most of the exercises. These Laboratory Reports are designed to guide and reinforce student learning and provide a convenient place for recording data. These reports include various types of review activities, tables for recoding observations and experimental results, and questions dealing with the analysis of such data.

As a result of these activities, students will increase their skills in gathering information by observation and experimentation. By completing all of the assessments in the Laboratory Reports, students will be able to determine if they accomplished all of the learning outcomes.

Digital Tools for Your Lab Course

McGraw-Hill Connect®

McGraw-Hill Connect allows instructors and students to use art and animations for assignments and lectures. A robust set of questions and activities are presented and aligned with the lab manual's exercises. As an instructor, you can edit existing questions and author entirely new problems. Track individual student performance—by question, assignment, or in relation to the class overall—with detailed grade reports. Instructors also have access to a variety of new resources, including assignable and gradable lab

Part 4, Staining and Observation of Microorganisms

- Exercises 11–18 on staining reactions have been reorganized and modified. Exercises have photographs in larger formats and some new photographs to show expected results. Also, each exercise now has its own set of results and questions, thus eliminating the necessity for students to record results and search for photos in later exercises.
- Exercise 15, Gram Staining, now includes new enlarged photomicrographs depicting gram-positive and gram-negative cells and align clearly with the procedure. More background on the history, importance, and theoretical basis of the Gram staining method has also been included. A corrected figure for the Gram stain steps is included as well. Procedures have slightly changed so that students have control bacteria on each slide to assist them in determining the success of their staining technique. The Laboratory Report has been enhanced, asking students to apply the concepts of cell shape and arrangement when evaluating their stained results.

Part 5, Culture Methods

- Enumeration of Bacteria: The Standard Plate Count, Exercise 21, now has photos of a set of serial dilution plates of a bacterial sample.

Part 6, Bacterial Viruses

- The steps in the infection of a bacterial cell by a bacteriophage have been revised in Exercise 23, Determination of a Bacteriophage Titer.
- Exercise 24, A One-Step Bacteriophage Growth Curve, includes major revisions that have been made to the background material and the procedure for enhanced student understanding and ease of implementation in the classroom. A figure of the one-step growth curve was added to the introductory material. Procedural figures were revised or deleted for clarity and alignment with changes to procedure. A graphing exercise and related analysis was added to the Laboratory Report.

Part 7, Environmental Influences and Control of Microbial Growth

- Exercise 27, Effects of Oxygen on Growth, has been revised to include the discussion of how oxygen influences growth and how it defines various classes of bacteria. Photographs showing the growth patterns of aerobes, anaerobes, microaerophiles, and facultative anaerobes in thioglycollate broth have replaced a diagram.
- A description of the organisms associated with human skin has been added to Exercise 33, Evaluation of Alcohol: Its Effectiveness as an Antiseptic.
- Antimicrobic Sensitivity Testing: The Kirby-Bauer Method, Exercise 34, has been updated with new information concerning health worker–acquired infections and the problem of antibiotic-resistant bacteria. New photographs of Kirby-Bauer plates showing sensitivity and resistance have been added, as well as photos showing how to measure the zone of inhibition.

Part 8, Identification of Unknown Bacteria

- Exercise 39, Physiological Characteristics: Oxidation and Fermentation Tests, now includes new photos of fermentation reactions (Durham tubes), the MRVP test, the citrate test, and the catalase.
- Added to Exercise 40, Physiological Characteristics: Hydrolytic and Degradative Reactions, are new photos of starch, casein, and fat hydrolysis.
- Physiological Characteristics: Multiple Test Media, Exercise 41, has new photos for SIM medium showing motility and hydrogen sulfide production. Enhanced photos of litmus milk reactions, including stormy fermentation, have also been added.
- The introductory material and separation outlines for Exercise 42, Use of *Bergey's Manual,* have been updated to reflect the current edition of Bergey's and the different volumes (both determinative and systematic). An additional challenge was added to this exercise aimed at teaching students how to use a table of test results and construct a flow chart to determine the identity of an unknown bacterium. A new lab report was added to this exercise for both the original procedure and the additional challenge.

Part 10, Diversity and Environmental Microbiology

- Exercise 47, Isolation of an Antibiotic Producer: The Streptomyces, includes the revision of the introductory section focusing on the role of the Streptomyces in antibiotic production. New photos of Streptomyces colonies on glycerol yeast extract agar have been added.

Part 13, Medical Microbiology

- Exercise 69, The Staphylococci: Isolation and Identification, includes new photos for Gram stain of staph, coagulase test, methyl green DNase test agar, and novobiocin sensitivity of Staphylococcus epidermidis.
- The Streptococci and Enterococci: Isolation and Identification, Exercise 70, includes a new photo of Gram stain of strep.

- Exercise 71, Gram-Negative Intestinal Pathogens, includes new photos of lactose fermenters on McConkey and Eosin methylene blue agar.
- A Synthetic Epidemic, Exercise 72, has two new figures added to the introductory material, highlighting the two different categories of epidemics and the concept of herd immunity. Procedure B and its corresponding lab report section were revised to illustrate the concept of herd immunity.

Part 14, Immunology and Serology

- Exercise 74, Slide Agglutination Test for *S. aureus*, has a new photo of the C-reactive protein agglutination test showing positive and negative results.

Acknowledgements

Our deepest gratitude to Judy Kaufman for her thorough review of the exercises and to Jill Kolodsick for her assistance in preparing the final draft of the manuscript and for her recommendations concerning the exercises.

We also wish to express our gratitude to Lisa Burgess for her excellent photographic contributions to the manual. The many new photos she provided will greatly improve the manual by providing clarity to many exercises.

The updates and improvements in this edition were guided by the helpful reviews and survey responses from the following instructors. Their input was critical to the decisions that shaped this edition.

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Basic Microbiology Laboratory Safety

Every student and instructor must focus on the need for safety in the microbiology laboratory. While the lab is a fascinating and exciting learning environment, there are hazards that must be acknowledged and rules that must be followed to prevent accidents and contamination with microbes. The following guidelines will provide every member of the laboratory section the information required to assure a safe learning environment.

Microbiological laboratories are special, often unique environments that may pose identifiable infectious disease risks to persons who work in or near them. Infections have been contacted in the laboratory throughout the history of microbiology. Early reports described laboratory-associated cases of typhoid, cholera, glanders, brucellosis, and tetanus, to name a few. Recent reports have documented laboratory-acquired cases in laboratory workers and health-care personnel involving *Bacillus anthracis, Bordetella pertussis, Brucella, Burkholderia pseudomallei, Campylobacter, Chlamydia,* and toxins from *Clostridium tetani, Clostridium botulinum,* and *Corynebacterium diphtheriae.* While we have a greater knowledge of these agents and antibiotics with which to treat them, safety and handling still remain primary issues.

The "Biohazard" symbol must be affixed to any container or equipment used to store or transport potentially infectious materials. Courtesy of the Centers for Disease Control.

The term "containment" is used to describe the safe methods and procedures for handling and managing microorganisms in the laboratory. An important laboratory procedure practiced by all microbiologists that will guarantee containment is **aseptic technique,** which prevents workers from contaminating themselves with microorganisms, ensures that others and the work area do not become contaminated, and also ensures that microbial cultures do not become unnecessarily contaminated with unwanted organisms. Containment involves personnel and the immediate laboratory and is provided by good microbiological technique and the use of appropriate safety equipment. Containment also guarantees that infectious agents do not escape from the laboratory and contaminate the environment external to the lab. Containment, therefore, relies on good microbiological technique and laboratory protocol as well as elements of laboratory design.

Biosafety Levels (BSL)

The recommended biosafety level(s) for handling microorganisms represent the potential of the agent to cause disease and the conditions under which the agent should be safely handled. The Centers for Disease Control classifies organisms into levels and sets guidelines for handling and safety measures required. These levels take into account many factors such as virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, and other factors. The recommended biosafety levels are as follows:

1. **BSL 1**—agents not known to cause disease in healthy adults; standard microbiological practices (SMP) apply; no safety equipment required; sinks required. Examples: *Bacillus subtilis, Micrococcus luteus.*
2. **BSL 2**—agents associated with human disease; standard microbiological practices apply plus limited access, biohazard signs, sharps precautions, and a biosafety manual required. Biosafety cabinet (BSC) used for aerosol/splash generating operations; lab coats, gloves, face protection required; contaminated waste is autoclaved.

All microorganisms used in the exercises in this manual are classified as BSL 1 or BSL 2. Examples: *Staphylococcus aureus, Streptococcus pyogenes.* **Note:** Although some of the organisms that students will culture and work with are classified as BSL 2, these organisms are laboratory strains that do not pose the same threat of infection as primary isolates of the same organism taken from patients in clinical samples. Hence, these laboratory strains can, in most cases, be handled using normal procedures and equipment found in the vast majority of student teaching laboratories. However, it should be emphasized that many bacteria are opportunistic pathogens, and therefore all microorganisms should be handled by observing proper techniques and precautions.

3. **BSL 3**—indigenous/exotic agents that may have serious or lethal consequences and with a potential for aerosol transmission. BSL 2 practices plus controlled access; decontamination of all waste and lab clothing before laundering; determination of baseline antibody titers to agents; biosafety cabinets used for all specimen manipulations; respiratory protection used as needed; physical separation from access corridors; double door access; negative airflow into the lab; exhaust air not recirculated. Examples: *Mycobacterium tuberculosis* and vesicular stomatitis virus (VSV).
4. **BSL 4**—dangerous/exotic agents of a life-threatening nature or unknown risk of transmission; BSL 3 practices plus clothing change before entering the laboratory; shower required before leaving the lab; all materials decontaminated on

exit; positive pressure personnel suit required for entry; separated/isolated building; dedicated air supply/exhaust and decontamination systems. Examples: Ebola and Lassa viruses.

Each of the biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed and the documented or suspected routes of transmission of the infectious agents. Common to all biosafety levels are standard practices, especially aseptic technique. Refer to the Biosafety Levels table on page xvi for a list of common organisms.

Standard Laboratory Rules and Practices

1. Students should store all books and materials not used in the laboratory in areas or receptacles designated for that purpose. Only necessary materials such as a lab notebook, the laboratory manual, and pen/pencil should be brought to the student work area.
2. Eating, drinking, chewing gum, and smoking are not allowed in the laboratory. Students must also avoid handling contact lenses or applying makeup while in the laboratory.
3. Safety equipment:
   a. Some labs will require that lab coats be worn in the laboratory at all times. Others may make this optional or not required. Lab coats can protect a student from contamination by microorganisms that he/she is working with and prevent contamination from stains and chemicals. At the end of the laboratory session, lab coats are usually stored in the lab in a manner prescribed by the instructor. Lab coats, gloves, and safety equipment should not be worn outside of the laboratory unless properly decontaminated first.
   b. You may be required to wear gloves while performing the lab exercises. This is especially important if you have open wounds. They protect the hands against contamination by microorganisms and prevent the hands from coming in direct contact with stains and other reagents.
   c. Face protection/safety glasses may be required by some instructors while you are performing experiments. Safety glasses can prevent materials from coming in contact with the eyes. They must be worn especially when working with ultraviolet light to prevent eye damage because they block out UV rays. If procedures involve the potential for splash/aerosols, face protection should be worn.
   d. Know the location of eye wash and shower stations in the event of an accident that requires the use of this equipment. Also know the location of first aid kits.
4. Sandals or open-toe shoes are not to be worn in the laboratory. Accidental dropping of objects or cultures could result in serious injury or infection.
5. Students with long hair should tie the hair back to avoid accidents when working with Bunsen burners/open flames. Long hair can also be a source of contamination when working with cultures.
6. Before beginning the activities for the day, work areas should be wiped down with the disinfectant that is provided for that purpose. Likewise, when work is finished for the day, the work area should be treated with disinfectant to ensure that any contamination from the exercise performed is destroyed. Avoid contamination of the work surface by not placing contaminated pipettes, loops/needles, or swabs on the work surface. Dispose of contaminated paper towels used for swabbing in the biohazard container.
7. Use extreme caution when working with open flames. The flame on a Bunsen burner is often difficult to see when not in use. Caution is imperative when working with alcohol and open flames. Alcohol is highly flammable, and fires can easily result when using glass rods that have been dipped in alcohol. **Always make sure the gas is turned off before leaving the laboratory.**
8. Any cuts or injuries on the hands must be covered with band-aids to prevent contamination. If you injure or cut yourself during the laboratory, notify the laboratory instructor immediately.
9. Pipetting by mouth is prohibited in the lab. All pipetting must be performed with pipette aids. Be especially careful when inserting glass pipettes into pipette aids as the pipette can break and cause a serious injury.
10. Know the location of exits and fire extinguishers in the laboratory.
11. Most importantly, read the exercise and understand the laboratory protocol before coming to laboratory. In this way you will be familiar with potential hazards in the exercise.
12. When working with microfuges, be familiar with their safe operation and make sure that all microfuge tubes are securely capped before centrifuging.
13. When working with electrophoresis equipment, follow the directions carefully to avoid electric shock.
14. If you have any allergies or medical conditions that might be complicated by participating in the laboratory, inform the instructor. Women who are pregnant should discuss the matter of enrolling in

the lab with their family physician and the laboratory instructor.
15. Unless directed to do so, do not subculture any unknown organisms isolated from the environment as they could be potential pathogens.
16. Avoid handling personal items such as cell phones, calculators, and cosmetics while performing the day's exercise.
17. You may be required to sign a safety agreement stating that you have been informed about safety issues and precautions and the hazardous nature of microorganisms that you may handle during the laboratory course.
18. Avoid wearing dangling jewelry to lab.

Disposal of Biological Wastes

Dispose of all contaminated materials properly and in the appropriate containers:

1. Biohazard containers—biohazard containers are to be lined with clear autoclave bags; disposable petri plates, used gloves, and any materials such as contaminated paper towels should be discarded in these containers; no glassware, test tubes, or sharp items are to be disposed of in biohazard containers.
2. Sharps containers—sharps, slides, coverslips, broken glass, disposable pipettes, and Pasteur pipettes should be discarded in these containers. If instructed to do so, you can discard contaminated swabs, wooden sticks, and microfuge tubes in the sharps containers.
3. Discard shelves, carts, bins, etc.—contaminated culture tubes and glassware used to store media and other glassware should be placed in these areas for decontamination and washing.
4. Trash cans—any noncontaminated materials, paper, or trash should be discarded in these containers. Under no circumstances should laboratory waste be disposed of in trash cans.

Discard other materials as directed by your instructor. This may involve placing materials such as slides contaminated with blood in disinfectant baths before these materials can be discarded.

Emergencies

Surface Contamination

1. Report all spills immediately to the laboratory instructor.
2. Cover the spill with paper towels and saturate the paper towels with disinfectant.
3. Allow the disinfectant to act for at least 20 minutes.
4. Remove any glass or solid material with forceps or scoop and discard the waste in an appropriate manner.

Personnel Contamination

1. Notify lab instructor.
2. Clean exposed area with soap/water, eye wash (eyes) or saline (mouth).
3. Apply first aid.

Biosafety Levels for Selected Infectious Agents

BIOSAFETY LEVEL (BSL)	TYPICAL RISK	ORGANISM
BSL 1	Not likely to pose a disease risk to healthy adults.	*Achromobacter denitrificans* *Alcaligenes faecalis* *Bacillus cereus* *Bacillus subtilis* *Corynebacterium pseudodiphtheriticum* *Enterococcus faecalis* *Micrococcus luteus* *Neisseria sicca* *Proteus vulgaris* *Pseudomonas aeruginosa* *Staphylococcus epidermidis* *Staphylococcus saprophyticus*
BSL 2	Poses a moderate risk to healthy adults; unlikely to spread throughout community; effective treatment readily available.	*Enterobacter aerogenes* *Escherichia coli* *Klebsiella pneumoniae* *Mycobacterium phlei* *Salmonella enterica var. Typhimurium* *Shigella flexneri* *Staphylococcus aureus* *Streptococcus pneumoniae* *Streptococcus pyogenes*
BSL 3	Can cause disease in healthy adults; may spread to community; effective treatment readily available.	*Blastomyces dermatitidis* *Chlamydia trachomatis* *Coccidioides immitis* *Coxiella burnetii* *Francisella tularensis* *Histoplasma capsulatum* *Mycobacterium bovis* *Mycobacterium tuberculosis* *Pseudomonas mallei* *Rickettsia canadensis* *Rickettsia prowazekii* *Yersinia pestis*
BSL 4	Can cause disease in healthy adults; poses a lethal risk and does not respond to vaccines or antimicrobial therapy.	Filovirus *Herpesvirus simiae* Lassa virus Marburg virus

Microorganisms Used or Isolated in the Lab Exercises in This Manual

ORGANISM	GRAM STAIN AND MORPHOLOGY	HABITAT	BSL	LAB EXERCISE
Alcaligenes faecalis ATCC 8750	Negative rod	Decomposing organic material, feces	1	29, 42
Azotobacter nigricans ATCC 35009	Negative rod	Soil, water	1	50
Azotobacter vinelandii ATCC 478	Negative rod	Soil, water	1	50
Bacillus mycoides ATCC 6462	Positive rod in chains	Soil	1	57
Bacillus coagulans ATCC 7050	Positive rod	Spoiled food, silage	1	63
Bacillus megaterium ATCC 14581	Positive rod	Soil, water	1	12, 13, 15, 16, 31, 62
Bacillus subtilis ATCC 23857	Positive rod	Soil, decomposing organic matter	1	27, 40
Candida glabrata ATCC 200918	Yeast	Human oral cavity	1	29
Chromobacterium violaceum ATCC 12472	Negative rod	Soil and water; opportunistic pathogen in humans	1	10
Citrobacter freundii ATCC 8090	Negative rod	Humans, animals, soil water; sewage opportunistic pathogen	1	71
Clostridium beijerinckii ATCC 25752	Positive rod	Soil	1	27
Clostridium sporogenes ATCC 3584	Positive rod	Soil, animal feces	1	27, 55, 63
Corynebacterium xerosis ATCC 373	Positive rods, club-shaped	Conjunctiva, skin	1	12
Desulfovibrio desulfuricans ATCC 25577	Negative, curved rods	Soil, sewage, water	1	54
Enterobacter aerogenes ATCC 13048	Negative rods	Feces of humans and animals	2	27, 39, 42, 59
Enterococcus faecalis ATCC 19433	Positive cocci in pairs, short chains	Water, sewage, soil, dairy products	2	27, 42, 70, 75
Enterococcus faecium ATCC 19434	Positive cocci in pairs, short chains	Feces of humans and animals	2	70, 75
Escherichia coli ATCC 11775	Negative rods	Sewage, intestinal tract of warm-blooded animals	2	9, 10, 15, 21, 23, 24, 25, 27, 28, 29, 30, 32, 34, 39, 40, 41, 42, 56, 57, 59, 62, 63, 65, 66, 67

Microorganisms Used or Isolated in the Lab Exercises in This Manual (continued)

ORGANISM	GRAM STAIN AND MORPHOLOGY	HABITAT	BSL	LAB EXERCISE
Geobacillus stearothermophilus ATCC 12980	Gram-positive rods	Soil, spoiled food	1	28, 63
Halobacterium salinarium ATCC 33170	Gives gram-negative reaction; rods	Salted fish, hides, meats	1	30
Klebsiella pneumoniae ATCC 13883	Negative rods	Intestinal tract of humans; respiratory and intestinal pathogen in humans	2	14, 42
Lactococcus lactis ATCC 19435	Positive cocci in chains	Milk and milk products	1	12
Micrococcus luteus ATCC 12698	Positive cocci that occur in pairs	Mammalian skin	1	10, 18, 32, 42
Moraxella catarrhalis ATCC 25238	Negative cocci that often occur in pairs with flattened sides	Pharynx of humans	1	15
Mycobacterium smegmatis ATCC 19420	Positive rods; may be Y-shaped or branched	Smegma of humans	1	17
Paracoccus denitrificans ATCC 17741	Negative spherical cells or short rods	Soil	1	51
Penicillium chrysogenum ATCC 10106	Filamentous fungus	Soil	1	57
Proteus vulgaris ATCC 29905	Negative rods	Intestines of humans, and animals; soil and polluted waters	1	18, 34, 40, 41, 42, 56, 71
Pseudomonas aeruginosa ATCC 10145	Negative rods	Soil and water; opportunistic pathogen in humans	1	15, 34, 35, 39, 42
Pseudomonas fluorescens ATCC 13525	Negative rods	Soil, water, spoiled food; clinical specimens	1	57
Saccharomyces cerevisiae ATCC 18824	Yeast	Fruit, used in beer, wine, and bread	1	29
Salmonella enterica subsp. enterica serovar *Typhimurium* ATCC 700720D-5	Negative rods	Most frequent agent of *Salmonella* gastroenteritis in humans	2	42, 71, 73
Serratia marcescens ATCC 13880	Negative rods	Opportunistic pathogen in humans	1	10, 28, 42, 72
Shigella flexneri ATCC 29903	Negative rods	Pathogen of humans	2	71
Staphylococcus aureus ATCC 12600	Positive cocci, irregular clusters	Skin, nose, GI tract of humans, pathogen	2	10, 12, 13, 15, 17, 26, 27, 29, 30, 31, 32, 34, 35, 39, 40, 41, 42, 55, 56, 57, 62, 69, 70, 74

Microorganisms Used or Isolated in the Lab Exercises in This Manual (continued)

ORGANISM	GRAM STAIN AND MORPHOLOGY	HABITAT	BSL	LAB EXERCISE
Staphylococcus epidermidis ATCC 14990	Positive cocci that occur in pairs and tetrads	Human skin, animals; opportunistic pathogen	1	42, 47, 69
Staphylococcus saprophyticus ATCC 15305	Positive cocci that occur singly and in pairs	Human skin; opportunistic pathogen in the urinary tract	1	69
Streptococcus agalactiae ATCC 13813	Positive cocci; occurs in long chains	Upper respiratory and vaginal tract of humans, cattle; pathogen	2	70, 75
Streptococcus bovis ATCC 33317	Positive cocci; pairs and chains	Cattle, sheep, pigs; occasional pathogen in humans	2	70, 75
Streptococcus dysagalactiae subsp. equisimilis ATCC 43078	Positive cocci in chains	Mastitis in cattle	2	70
Streptococcus mitis ATCC 49456	Positive cocci in pairs and chains	Oral cavity of humans	2	70
Streptococcus mutans ATCC 25175D-5	Positive cocci in pairs and chains	Tooth surface of humans, causes dental caries	2	70
Streptococcus pneumoniae ATCC 33400D-5	Positive cocci in pairs	Human pathogen	2	70
Streptococcus pyogenes ATCC 12344	Positive cocci in chains	Human respiratory tract; pathogen	2	70, 75
Streptococcus salivarius ATCC 19258	Positive cocci in short and long chains	Tongue and saliva	2	70
Thermoanaerobacterium thermosaccharolyticum ATCC 7956	Negative rods; single cells or pairs	Soil, spoiled canned foods	1	63

Contents

Indicates a LearnSmart Lab™ activity is available for all or part of this exercise. For more information, visit mhhe.com/lslabsmicro.