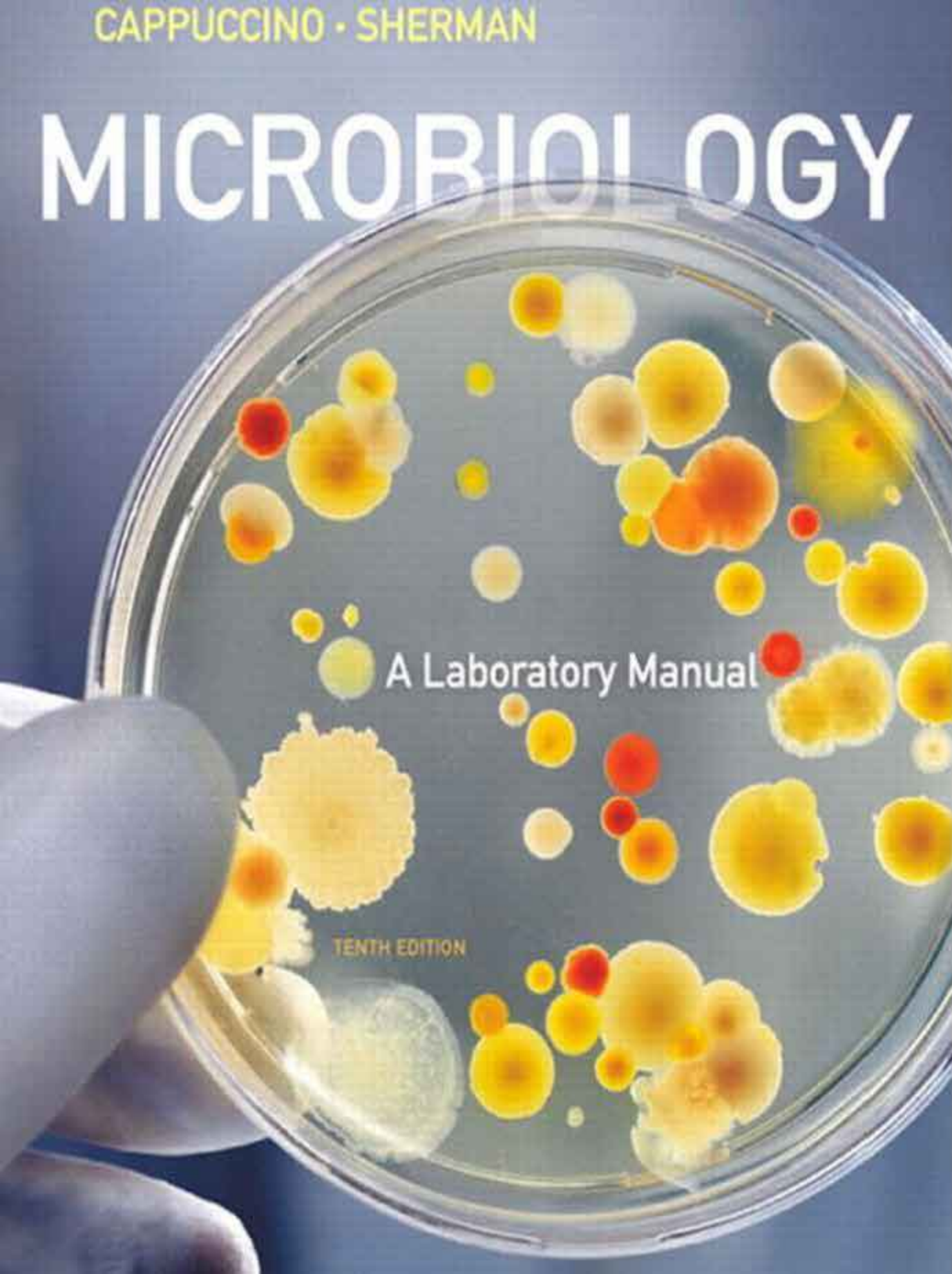
CAPPUCCINO · SHERMAN

MICROBIOLOGY



A Laboratory Manual

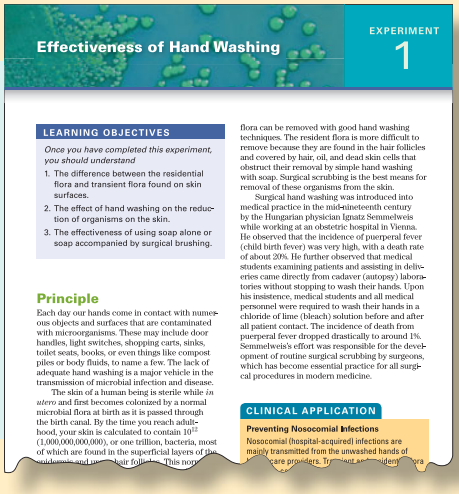
TENTH EDITION

New Features Make the Micro Lab More Engaging and Applicable to Real Life

Two newly added features in the Tenth Edition of *Microbiology: A Laboratory Manual* speak directly to student interest and engagement: A new experiment on hand washing and a new Clinical Applications feature that appears in each experiment.

NEW! Hand-Washing Experiment

A brand-new first experiment emphasizes the importance of effective hand washing and helps introduce microbiology in an engaging way. By learning about the presence of microorganisms on their own hands, this experiment gives students a practical understanding of why aseptic techniques in the lab are so important. A fun, practical lab, this experiment also gets students excited about the course to come.


EXPERIMENT 1

Effectiveness of Hand Washing

LEARNING OBJECTIVES

Once you have completed this experiment, you should understand

1. The difference between the residential flora and transient flora found on skin surfaces.
2. The effect of hand washing on the reduction of organisms on the skin.
3. The effectiveness of using soap alone or soap accompanied by surgical brushing.

Principle

Each day our hands come in contact with numerous objects and surfaces that are contaminated with microorganisms. These may include door handles, light switches, shopping carts, sinks, toilet seats, books, or even things like compost piles or body fluids, to name a few. The lack of adequate hand washing is a major vehicle in the transmission of microbial infection and disease.

The skin of a human being is sterile while *in utero* and first becomes colonized by a normal microbial flora at birth as it is passed through the birth canal. By the time you reach adulthood, your skin is calculated to contain 10^{12} (1,000,000,000,000), or one trillion, bacteria, most of which are found in the superficial layers of the [illegible] This normal flora can be removed with good hand washing techniques. The resident flora is more difficult to remove because they are found in the hair follicles and covered by hair, oil, and dead skin cells that obstruct their removal by simple hand washing with soap. Surgical scrubbing is the best means for removal of these organisms from the skin.

Surgical hand washing was introduced into medical practice in the mid-nineteenth century by the Hungarian physician Ignatz Semmelweis while working at an obstetric hospital in Vienna. He observed that the incidence of puerperal fever (child birth fever) was very high, with a death rate of about 20%. He further observed that medical students examining patients and assisting in deliveries came directly from cadaver (autopsy) laboratories without stopping to wash their hands. Upon his insistence, medical students and all medical personnel were required to wash their hands in a chloride of lime (bleach) solution before and after all patient contact. The incidence of death from puerperal fever dropped drastically to around 1%. Semmelweis's effort was responsible for the development of routine surgical scrubbing by surgeons, which has become essential practice for all surgical procedures in modern medicine.

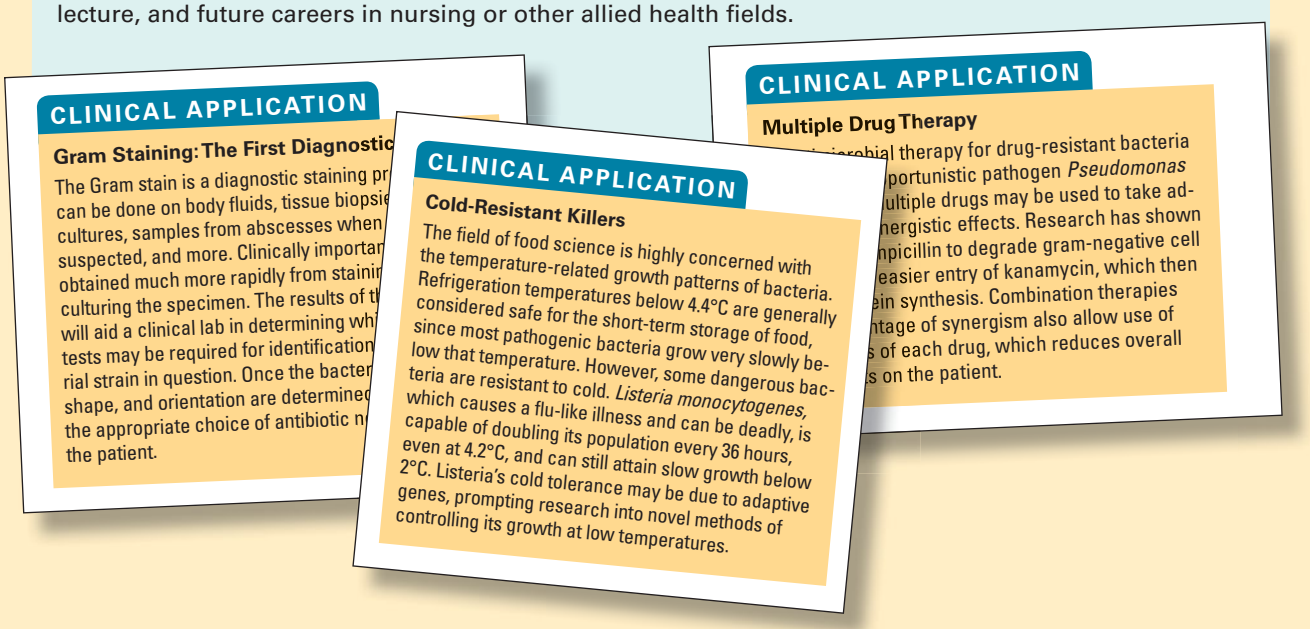
CLINICAL APPLICATION

Preventing Nosocomial Infections

Nosocomial (hospital-acquired) infections are mainly transmitted from the unwashed hands of [illegible]

NEW! Clinical Applications

Students often wonder how learning microbiological lab techniques will help them in their future careers. New Clinical Applications added to each experiment make the connection between lab, lecture, and future careers in nursing or other allied health fields.


CLINICAL APPLICATION

Gram Staining: The First Diagnostic

The Gram stain is a diagnostic staining pr
can be done on body fluids, tissue biopsie
cultures, samples from abscesses when
suspected, and more. Clinically importan
obtained much more rapidly from stainin
culturing the specimen. The results of t
will aid a clinical lab in determining wh
tests may be required for identification
rial strain in question. Once the bacter
shape, and orientation are determined
the appropriate choice of antibiotic n
the patient.

CLINICAL APPLICATION

Cold-Resistant Killers

The field of food science is highly concerned with the temperature-related growth patterns of bacteria. Refrigeration temperatures below 4.4°C are generally considered safe for the short-term storage of food, since most pathogenic bacteria grow very slowly below that temperature. However, some dangerous bacteria are resistant to cold. *Listeria monocytogenes*, which causes a flu-like illness and can be deadly, is capable of doubling its population every 36 hours, even at 4.2°C, and can still attain slow growth below 2°C. Listeria's cold tolerance may be due to adaptive genes, prompting research into novel methods of controlling its growth at low temperatures.

CLINICAL APPLICATION

Multiple Drug Therapy

[illegible]ial therapy for drug-resistant bacteria
portunistic pathogen *Pseudomonas*
ultiple drugs may be used to take ad-
nergistic effects. Research has shown
npicillin to degrade gram-negative cell
easier entry of kanamycin, which then
in synthesis. Combination therapies
ntage of synergism also allow use of
s of each drug, which reduces overall
s on the patient.

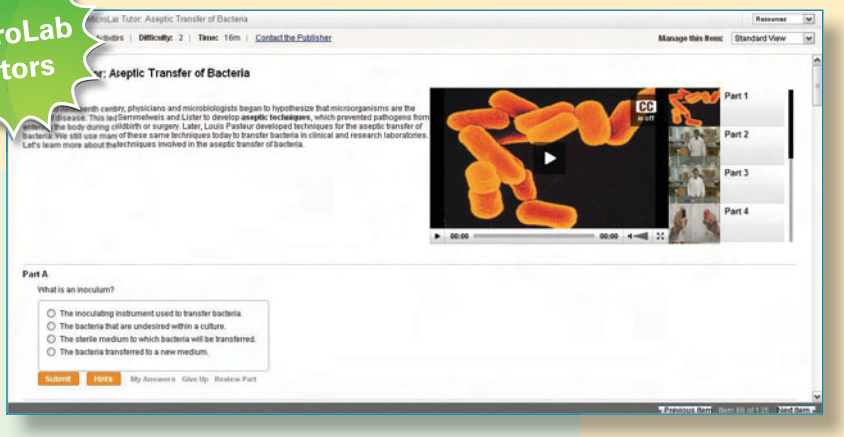
NEW! Photos of Protozoa, Yeast, and Fungi

Parts 6 and 7 of the manual now contain even more photos and micrographs of protozoa, yeast, and fungi to help students visualize these important organisms in the lab.

MasteringMicrobiology® Takes the Microbiology Laboratory Course to the Next Level

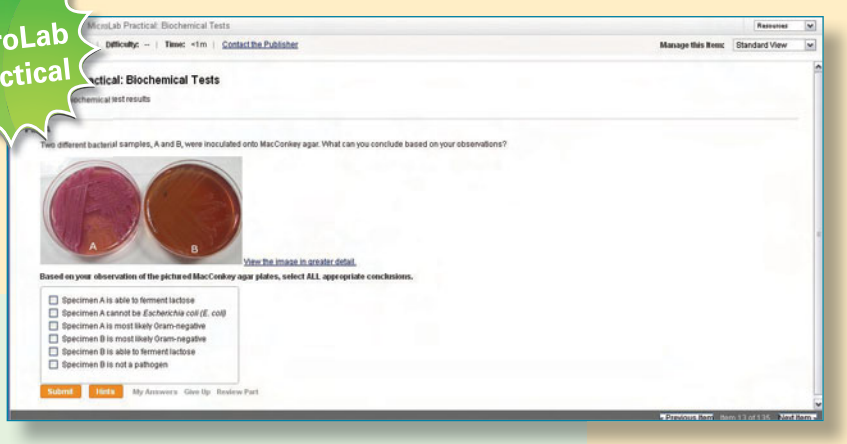
MasteringMicrobiology® The Mastering online homework, assessment, and tutorial system delivers self-paced tutorials that provide students with individualized coaching set to the instructor's course objectives. MasteringMicrobiology® helps students arrive better prepared for lecture and lab with practice quizzes, coaching activities, tutorials, and more. Instructors can now assign MicroLab Tutors, MicroLab Practicals, Lab Technique Videos, and Lab-specific Quizzes within the MasteringMicrobiology item library. Students can access the MicroLab Tutors, MicroLab Practicals and Lab Technique Videos for review and self-study in the StudyArea of MasteringMicrobiology.

MicroLab Tutors



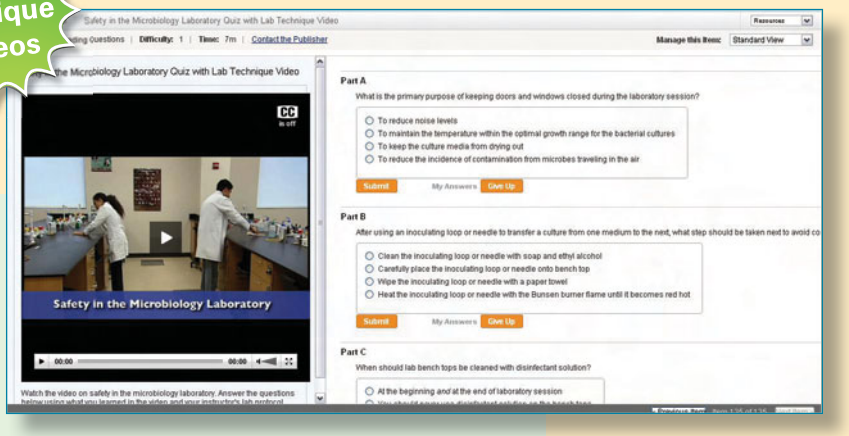
MicroLab Tutors help instructors and students get the most out of lab time by providing additional opportunities to reinforce lab concepts and techniques. MicroLab Tutors introduce important lab techniques and include each technique's background, purpose, and clinical applications before walking students through the procedure itself. Using a combination of micrographs, video footage in the lab, and, for select topics, 3D molecular animations, MicroLab Tutors help students visualize important lab techniques and processes and make the connection between lecture and lab.

MicroLab Practical



MicroLab Practical assessments give students extra practice in observing and analyzing test results–putting knowledge into practice. These Lab Practical questions give users an extra opportunity to analyze and interpret important lab tests, procedures, and results in order to get ready for the lab practical!

Lab Technique Videos



Lab Technique Videos are 3–5 minute videos, demonstrating specific lab techniques. These videos cover important procedures, such as aseptic technique, Gram staining, and smear preparation. The videos and their associated assessments help students prepare for the wet lab and allow them to review techniques on their own time.

Lab-Specific Quizzes

Lab-Specific Quizzes are newly created for each experiment in the manual. A total of ten multiple-choice and true/false questions per experiment allow instructors to quiz students on microbiological principles, lab techniques, and the theory behind lab procedures. Each quiz question is tied to a Learning Objective for the lab.

What Instructors are Saying

"These are the types of lab questions I am looking for. Hallelujah!"

▶ **Pele Rich**
North Hennepin Community College

"I think they are really great!! It puts things in a place where the students can observe firsthand what they should be seeing. I definitely would use them."

▶ **Tanya Crider**
East Mississippi Community College

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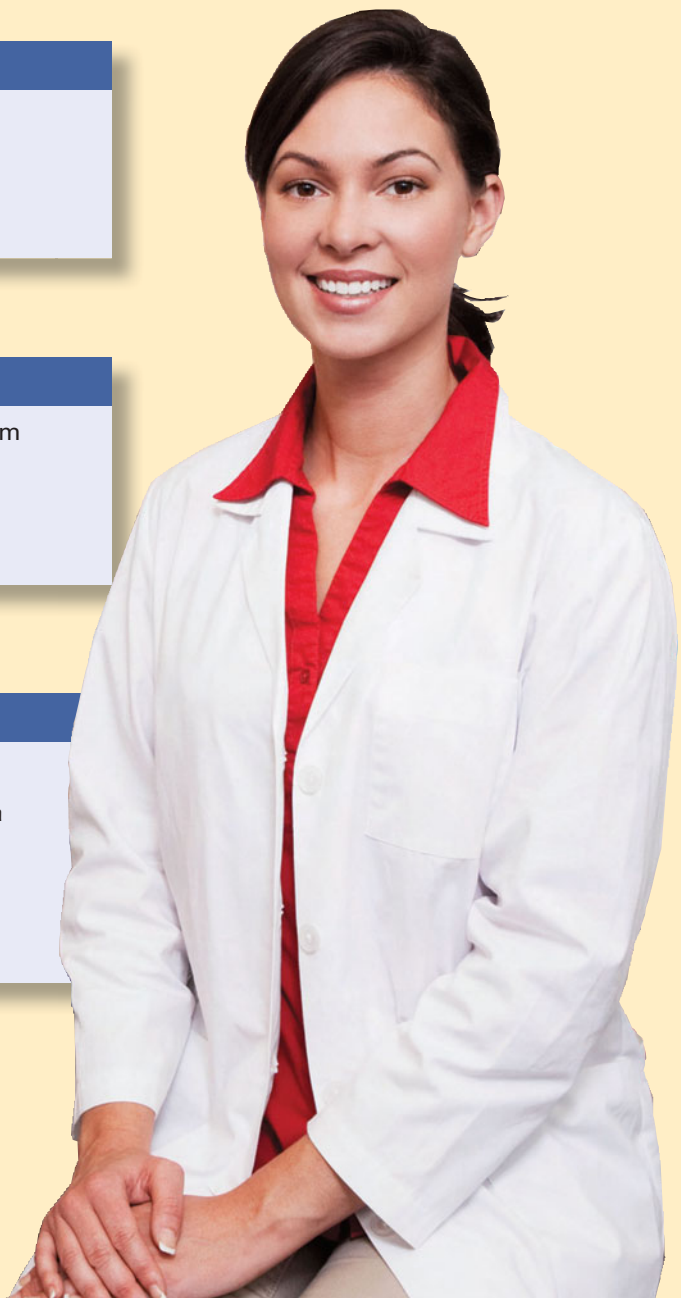
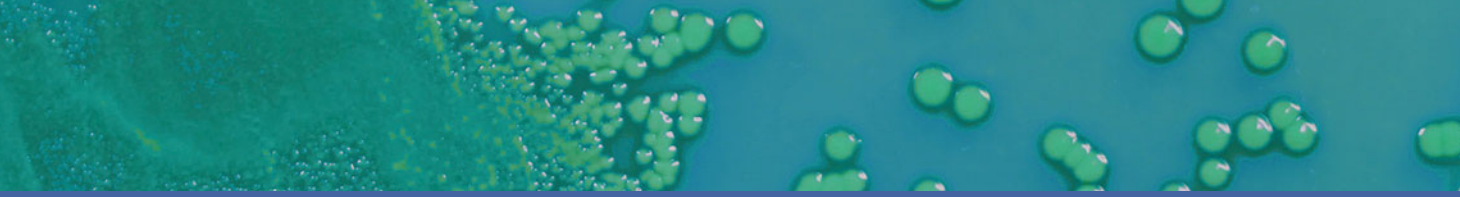
- Once you decide to adopt, use your automatically generated custom ISBN to place an order at your campus store.





MICROBIOLOGY

A LABORATORY MANUAL

TENTH EDITION

James G. Cappuccino

SUNY Rockland Community College

Natalie Sherman

SUNY Rockland Community College

PEARSON

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Contents

Dedication

1932–2001

It is with great pride that I dedicate this book to the memory of Natalie Sherman. She was my friend, colleague, and coauthor for 32 years. Her passion for teaching was only exceeded by her ability to teach her students well. They have become the beneficiaries of her unique talent.

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Preface

Microbiology is a dynamic science. It is constantly evolving as more information is added to the continuum of knowledge, and as microbiological techniques are rapidly modified and refined. The tenth edition of *Microbiology: A Laboratory Manual* continues to provide a blend of traditional methodologies with more contemporary procedures to meet the pedagogical needs of all students studying microbiology. As in previous editions, this laboratory manual provides a wide variety of critically selected and tested experiments suitable for undergraduate students in allied health programs, as well as elementary and advanced general microbiology courses.

Our Approach

This laboratory manual is designed to guide students in the development of manipulative skills and techniques essential for understanding the biochemical structure and function of a single cell. Its main goal is to encourage students to apply these laboratory skills in the vocational field of applied microbiology and allied health or to further pursue the study of life at the molecular level.

In this manual, comprehensive introductory material is given at the beginning of each major area of study, and specific explanations and detailed directions precede each experiment. This approach augments, enhances, and reinforces course lectures, enabling students to comprehend more readily the concepts and purposes of each experiment. This also provides a review aid if the laboratory and lecture sections are not taught concurrently. The manual should also reduce the time required for explanations at the beginning of each laboratory session and thus make more time available for performing the experiments. Finally, care has been taken to design experimental procedures so that the supplies, equipment, and instrumentation commonly found in undergraduate institutions will suffice for their successful execution.

Organization

This manual consists of 75 experiments arranged into 16 parts. The experiments progress from those that are basic and introductory, requiring minimal manipulations, to those that are more complex, requiring more sophisticated skills. The format of each experiment is intended to facilitate presentation of the material by the instructor and to maximize the learning experience. To this end, each experiment is designed as follows:

Learning Objectives

This introductory section defines the specific principles and/or techniques to be mastered.

Principle

This is an in-depth discussion of the microbiological concept or technique and the specific experimental procedure.

Clinical Application

Clinical or medical applications that appear within each experiment help students connect what they are learning in lecture with what they are doing in the lab. For students who intend to have careers as nurses or in other allied health fields, Clinical Applications explain the relevance of each lab technique to their career plans.

At the Bench

This section signals the beginning of the experiment, and includes the materials, notes of caution, and procedural instructions—all of the things students will need to know at the bench, during the course of the experiment.

Materials

This comprehensive list helps students and instructors prepare for each laboratory session. Materials appear under one of the following headings:

Cultures These are the selected test organisms that have been chosen to demonstrate effectively the experimental principle or technique under study. The choice is also based on their ease of cultivation and maintenance in stock culture. A complete listing of the experimental cultures and prepared slides is presented in Appendix 6.

Media These are the specific media and their quantities per designated student group. Appendix 3 lists the composition and method of preparation of all the media used in this manual.

Reagents These include biological stains as well as test reagents. The chemical composition and preparation of the reagents are presented in Appendices 4 and 5.

Equipment Listed under this heading are the supplies and instrumentation that are needed during the laboratory session. The suggested equipment was selected to minimize expense while reflecting current laboratory technique.

Procedure

This section provides explicit instructions, augmented by diagrams, that aid in the execution and interpretation of the experiment.

A caution icon has been placed in experiments that may use **potentially pathogenic materials**. The instructor may wish to perform some of these experiments as demonstrations.

Lab Report

These tear-out sheets, located at the end of each experiment, facilitate interpretation of data and subsequent review by the instructor. The *Observations and Results* portion of the report provides tables for recording observations and results, and helps the students draw conclusions from and interpret their data. The *Review Questions* aid the instructor in determining the student's ability to understand the experimental concepts and techniques. Questions that call for more critical thinking are indicated by the light bulb icon.

New to the Tenth Edition

For this tenth edition, the primary aim was to build upon and enrich the student experience. The changes described below are intended to impart the relevance of microbiological lab techniques to student career goals, and to enhance student understanding and performance of each of the microbiological procedures.

New Clinical Applications

The tenth edition includes new Clinical Applications added to each experiment that help students understand why they're learning individual techniques and connect the lab and lecture to students' future careers as nurses and in other allied health fields. With topics that vary from diagnosing lung infections to selection of effective antibiotics and beyond, clinical applications make the experiments come alive to students.

New Experiment 1: Effectiveness of Hand Washing

This experiment reinforces the importance of appropriate hand washing and introduces students to microbiology in a fun, easy way. By understanding that microorganisms thrive on their hands, students are also shown the importance of aseptic techniques, both to protect their health and preserve the value of the experiments they are performing.

New Quiz Questions in *Mastering Microbiology*®

Each experiment in the tenth edition is now accompanied by assignable, automatically graded quiz questions in MasteringMicrobiology®. Each Experiment includes 10 quiz questions correlated to the lab manual Learning Objectives for fast, convenient tracking of course outcomes in the MasteringMicrobiology gradebook.

Additional Protozoa, Fungi, and Yeast Photos Added

Based on reviewer requests, the tenth edition now includes nine new full-color photos and micrographs of protozoa, fungi, and yeast that can be found in Parts 6 and 7 of the manual.

New "Additional Readings" in the Instructor Guide

Additional reading research articles for each experiment have been added to the Instructor Guide. Articles are relevant to the experiment being performed and are ideal recommendations for further exploration.

Updates and Revisions

Throughout the manual, updates and revisions have been made to background information, terminology, equipment, and procedural techniques, including the following:

- Added a new procedure and photo for the slide method of the catalase test in Experiment 30.
- Added a new procedure for the filter paper method of the oxidase test in Experiment 31.
- New Observations and Results table in the Lab Report for Experiment 32 covers all experimental organisms.
- Updated photos of *Plasmodium vivax*, *Trypanosoma gambiense*, and *Giardia intestinalis* in Experiment 35.
- Added a new photo showing the minimum inhibitory concentration tube set-up in Experiment 45.
- Updated photo of the development of colonies on a membrane filter plate in Experiment 50.
- Figure 58.3 was altered to agree more precisely with the experimental bacterial transformation procedure.
- Deleted the Agglutination Reaction: Mono-Test for Infectious Mononucleosis from the ninth edition in order to make room for the new hand-washing experiment.

Instructor Resources

The *Instructor Guide* (ISBN 0-321-86365-8) is a valuable teaching aid for instructors. It has been updated to reflect changes in the main text, and provides the following:

- Laboratory safety protocol for the instructional staff
- Laboratory safety protocol for the technical staff
- New Additional Reading research articles for each experiment
- For each experiment: detailed lists of required materials, procedural points to emphasize, suggestions for optional procedural additions or modifications, helpful tips for preparing or implementing each experiment, and answers to the Review Questions in the student manual
- Appendices with the formulas for the preparation of all media, test reagents, and microbiological stains, as well as the microorganisms required for the performance of each procedure

Acknowledgments

I wish to express my sincere gratitude to the following instructors for their reviews of the tenth edition. Their comments and direction contributed greatly to the tenth edition.

Cynthia Anderson, *Mt. San Antonio College*
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Tanya Crider, *East Mississippi Community College*
Donald Deters, *Bowling Green State University*
Srinivasan Durairaj, *MacMurray College*
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Ralph Fleming, *Burlington County College*
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Jane McLaughlin, *Orange Coast College*
Oluwatoyin Osunsanya, *Muskingum University*
Adam Parks, *Shepherd University*
Diane Pelletier, *Green River Community College*
Stacy Pfluger, *Angelina College*
Nahed Salama, *SUNY Rockland Community College*
George Shiflet, *Wofford College*
Amy Sprenkle, *Salem State University*
Douglas Stemke, *University of Indianapolis*
Chad Welsh, *Lindenwood University*

I would like to express my sincere gratitude to Dr. Chad Welsh at Lindenwood University and Dr. James Yount at Brevard Community College for reviewing, editing, and writing Clinical Applications for the new edition. Their help made this exciting new feature even stronger. Thanks also to Dr. Welsh for providing the Additional Readings for the Instructor Guide. I am grateful for the work of Dr. Brent Sellinger at the University of Lethbridge. His fine photography and expert experimental setup will be valuable learning tools for students for many years to come.

I wish to extend my appreciation to the staff at Pearson Education, whose expertise and technical skills have guided me over many years. Marie Beaugureau, project editor, provided invaluable direction in this new edition; her dedication to the highest standards has left its mark on every page of the manual. In addition, I would like to thank Kelsey Churchman, acquisitions editor, Megan Power, production coordinator, and Cheryl Wilson, project manager. I also wish to express my gratitude to the microbiology laboratory technicians at SUNY Rockland Community College—Ms. Joan Grace, who early on performed all the experiments to ensure their success when repeated by the students, and Ms. Roz Wehrman, who followed in Joan's footsteps. Last, but certainly not least, I would like to acknowledge Ms. Allyson Cerreon for her assistance with the hand washing experiment.

James G. Cappuccino

Laboratory Safety

General Rules and Regulations

A rewarding laboratory experience demands strict adherence to prescribed rules for personal and environmental safety. The former reflects concern for your personal safety in terms of avoiding laboratory accidents. The latter requires that you maintain a scrupulously clean laboratory setting to prevent contamination of experimental procedures by microorganisms from exogenous sources.

Because most microbiological laboratory procedures require the use of living organisms, an integral part of all laboratory sessions is the use of aseptic techniques. Although the virulence of microorganisms used in the academic laboratory environment has been greatly diminished because of their long-term maintenance on artificial media, *all microorganisms should be treated as potential pathogens* (organisms capable of producing disease). Thus, microbiology students must develop aseptic techniques (free of contaminating organisms) in the preparation of pure cultures that are essential in the industrial and clinical marketplaces.

The following basic steps should be observed at all times to reduce the ever-present microbial flora of the laboratory environment.

1. Upon entering the laboratory, place coats, books, and other paraphernalia in specified locations—never on bench tops.
2. Keep doors and windows closed during the laboratory session to prevent contamination from air currents.
3. At the beginning and termination of each laboratory session, wipe bench tops with a disinfectant solution provided by the instructor.
4. Do not place contaminated instruments, such as inoculating loops, needles, and pipettes, on bench tops. Loops and needles should be sterilized by incineration, and pipettes should be disposed of in designated receptacles.
5. On completion of the laboratory session, place all cultures and materials in the disposal area as designated by the instructor.
6. Rapid and efficient manipulation of fungal cultures is required to prevent the dissemination of their reproductive spores in the laboratory environment.

To prevent accidental injury and infection of yourself and others, observe the following regulations:

1. Wash your hands with liquid detergent, rinse with 95% ethyl alcohol, and dry them with paper towels upon entering and prior to leaving the laboratory.
2. Always use the appropriate safety equipment as determined by your instructor:
   - **a.** A laboratory coat or apron may be necessary while working in the laboratory. Lab coats protect clothing from contamination or accidental discoloration by staining solutions.
   - **b.** You may be required to wear gloves while performing the lab exercises. Gloves shield your hands from contamination by microorganisms. They also prevent the hands from coming in direct contact with stains and other reagents.
   - **c.** Masks and safety goggles may be required to prevent materials from coming in contact with your eyes.
3. Wear a paper cap or tie back long hair to minimize its exposure to open flames.
4. Wear closed shoes at all times in the laboratory setting.
5. Never apply cosmetics or insert contact lenses in the laboratory.
6. Do not smoke, eat, or drink in the laboratory. These activities are absolutely prohibited.
7. Carry cultures in a test-tube rack when moving around the laboratory. Likewise, keep cultures in a test-tube rack on the bench tops when not in use. This serves a dual purpose: to prevent accidents and to avoid contamination of yourself and the environment.

8. Never remove media, equipment, or especially, *microbial cultures* from the laboratory. Doing so is absolutely prohibited.
9. Immediately cover spilled cultures or broken culture tubes with paper towels and then saturate them with disinfectant solution. After 15 minutes of reaction time, remove the towels and dispose of them in a manner indicated by the instructor.
10. Report accidental cuts or burns to the instructor immediately.
11. Never pipette by mouth any broth cultures or chemical reagents. Doing so is strictly prohibited. Pipetting is to be carried out with the aid of a mechanical pipetting device only.
12. Do not lick labels. Use only self-stick labels for the identification of experimental cultures.
13. Speak quietly and avoid unnecessary movement around the laboratory to prevent distractions that may cause accidents.

The following specific precautions must be observed when handling body fluids of unknown origin due to the possible transmission of the HIV and hepatitis B viruses in these test specimens.

1. Wear disposable gloves during the manipulation of test materials such as blood, serum, and other body fluids.
2. Immediately wash hands if contact with any of these fluids occurs and also upon removal of the gloves.
3. Wear masks, safety goggles, and laboratory coats if an aerosol might be formed or splattering of these fluids is likely to occur.
4. Decontaminate spilled body fluids with a 1:10 dilution of household bleach, covered with paper toweling, and allowed to react for 10 minutes before removal.
5. Place test specimens and supplies in contact with these fluids into a container of disinfectant prior to autoclaving.

I have read the above laboratory safety rules and regulations and agree to abide by them.

Name: _____ Date: _____

Student Preparation for Laboratory Sessions

The efficient performance of laboratory exercises mandates that you attend each session fully prepared to execute the required procedures. Read the assigned experimental protocols to effectively plan and organize the related activities. This will allow you to maximize use of laboratory time.

Preparation of Experimental Materials

Microscope Slides: Meticulously clean slides are essential for microscopic work. Commercially precleaned slides should be used for each microscopic slide preparation. However, wipe these slides with dry lens paper to remove dust and finger marks prior to their use. With a glassware marking pencil, label one end of each slide with the abbreviated name of the organism to be viewed.

Labeling of Culture Vessels: Generally, microbiological experiments require the use of a number of different test organisms and a variety of culture media. To ensure the successful completion of experiments, organize all experimental cultures and sterile media at the start of each experiment. Label culture vessels with non–water-soluble glassware markers and/or self-stick labels prior to their inoculation. The labeling on each of the experimental vessels should include the name of the test organism, the name of the medium, the dilution of sample (if any), your name or initials, and the date. *Place labeling directly below the cap of the culture tube.* When labeling Petri dish cultures, only the name of the organism(s) should be written on the bottom of the plate, close to its periphery, to prevent obscuring observation of the results. The additional information for the identification of the culture should be written on the cover of the Petri dish.

Inoculation Procedures

Aseptic techniques for the transfer or isolation of microorganisms, using the necessary transfer instruments, are described fully in the experiments in Part 1 of the manual. Technical skill will be acquired through repetitive practice.

Inoculating Loops and Needles: It is imperative that you incinerate the entire wire to ensure absolute sterilization. The shaft should also be briefly passed through the flame to remove any dust or possible contaminants. To avoid killing the cells and splattering the culture, cool the inoculating wire by tapping the inner surface of the culture tube or the Petri dish cover prior to obtaining the inoculum, or touch the edge of the medium in the plate.

When performing an aseptic transfer of microorganisms, a minute amount of inoculum is required. If an agar culture is used, touch only a single area of growth with the inoculating wire to obtain the inoculum. *Never drag the loop or needle over the entire surface, and take care not to dig into the solid medium.* If a broth medium is used, first tap the bottom of the tube against the palm of your hand to suspend the microorganisms. *Caution: Do not tap the culture vigorously as this may cause spills or excessive foaming of the culture, which may denature the proteins in the medium.*

Pipettes: Use only sterile, disposable pipettes or glass pipettes sterilized in a canister. The practice of *pipetting by mouth has been discontinued* to eliminate the possibility of autoinfection by accidentally imbibing the culture or infectious body fluids. Instead, use a mechanical pipetting device to obtain and deliver the material to be inoculated.

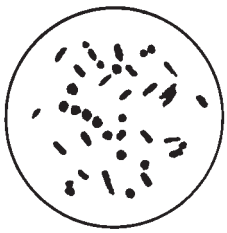
Incubation Procedure

Microorganisms exhibit a wide temperature range for growth. However, for most used in this manual, optimum growth occurs at 37°C over a period of 18 to 24 hours. Unless otherwise indicated in specific exercises, incubate all cultures under the conditions cited above. Place culture tubes in a rack for incubation. Petri dishes may be stacked; however, they *must always be incubated in an inverted position (top down)* to prevent water condensation from dropping onto the surface of the culture medium. This excess moisture could allow the spread of the microorganisms on the surface of the culture medium, producing confluent rather than discrete microbial growth.

Procedure for Recording Observations and Results

The accurate accumulation of experimental data is essential for the critical interpretation of the observations upon which the final results will be based. To achieve this end, it is imperative that you complete all the preparatory readings that are necessary for your understanding of the basic principles underlying each experiment. Meticulously record all the observed data in the Lab Report of each experiment.

In the experiments that require drawings to illustrate microbial morphology, it will be advantageous to depict shapes, arrangements, and cellular structures enlarged to 5 to 10 times their actual microscopic size, as indicated by the following illustrations. For this purpose a number 2 pencil is preferable. Stippling may be used to depict different aspects of cell structure (e.g., endospores or differences in staining density).



Microscopic drawing

Enlarged drawing

Review Questions

The review questions are designed to evaluate the student's understanding of the principles and the interpretations of observations in each experiment. Completion of these questions will also serve to reinforce many of the concepts that are discussed in the lectures. At times, this will require the use of ancillary sources such as textbooks, microbiological reviews, or abstracts. The designated critical-thinking questions are designed to stimulate further refinement of cognitive skills.

Procedure for Termination of Laboratory Sessions

1. Return all equipment, supplies, and chemical reagents to their original locations.
2. Neatly place all capped test tube cultures and closed Petri dishes in a designated collection area in the laboratory for subsequent autoclaving.
3. Place contaminated materials, such as swabs, disposable pipettes, and paper towels, in a biohazard receptacle prior to autoclaving.
4. Carefully place hazardous biochemicals, such as potential carcinogens, into a sealed container and store in a fume hood prior to their disposal according to the institutional policy.
5. Wipe down table tops with recommended disinfectant.
6. Wash hands before leaving the laboratory.

PART 1

Basic Laboratory Techniques for Isolation, Cultivation, and Cultural Characterization of Microorganisms

LEARNING OBJECTIVES

Once you have completed the experiments in this section, you should be familiar with

1. The types of laboratory equipment and culture media needed to develop and maintain pure cultures.
2. The types of microbial flora that live on the skin and the effect of hand washing on them.
3. The concept of aseptic technique and the procedures necessary for successful subculturing of microorganisms.
4. Streak-plate and spread-plate inoculation of microorganisms in a mixed microbial population for subsequent pure culture isolation.
5. Cultural and morphological characteristics of microorganisms grown in pure culture.

Introduction

Microorganisms are ubiquitous. They are found in soil, air, water, food, sewage, and on body surfaces. In short, every area of our environment is replete with them. The microbiologist separates these mixed populations into individual species for study. A culture containing a single unadulterated species of cells is called a **pure culture.** To isolate and study microorganisms in pure culture, the microbiologist requires basic laboratory apparatus and the application of specific techniques, as illustrated in **Figure P1.1**.

Media

The survival and continued growth of microorganisms depend on an adequate supply of nutrients and a favorable growth environment. For survival, most microbes must use soluble low-molecular-weight substances that are frequently derived from the enzymatic degradation of complex nutrients. A solution containing these nutrients is a **culture medium.** Basically, all culture media are liquid, semisolid, or solid. A liquid medium lacks a solidifying agent and is called a **broth medium.** A broth medium supplemented with a solidifying agent called **agar** results in a solid or semisolid medium. Agar, an extract of seaweed, is a complex carbohydrate composed mainly of galactose, and is without nutritional value. Agar serves as an excellent solidifying agent because it liquefies at 100°C and solidifies at 40°C. Because of these properties, organisms, especially pathogens, can be cultivated at temperatures of 37.5°C or slightly higher without fear of the medium liquefying. A completely solid medium requires an agar concentration of about 1.5 to 1.8%. A concentration of less than 1% agar results in a **semisolid medium.** A solid medium has the advantage that it presents a hardened surface on which microorganisms can be grown using specialized techniques for the isolation of discrete colonies. Each colony is a cluster of cells that originates from the multiplication of a single cell and

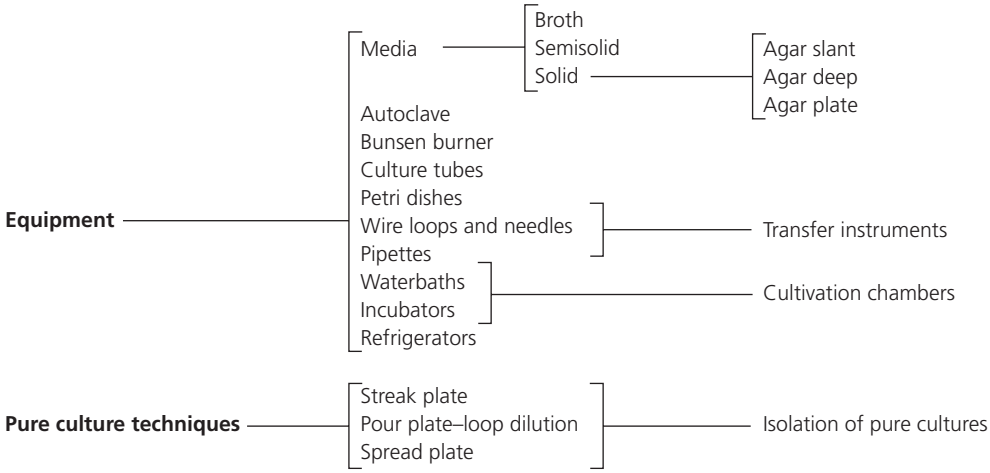
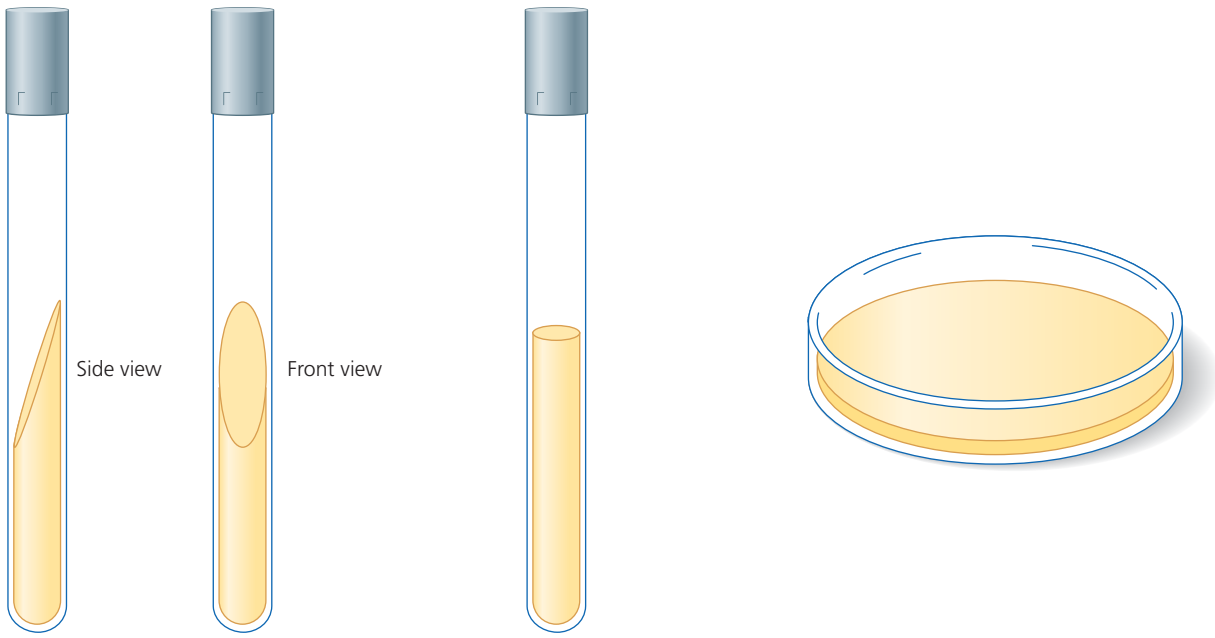


Figure P1.1 **Laboratory apparatus and culture techniques**

represents the growth of a single species of microorganism. Such a defined and well-isolated colony is a pure culture. Also, while in the liquefied state, solid media can be placed in test tubes, which are then allowed to cool and harden in a slanted position, producing **agar slants.** These are useful for maintaining pure cultures. Similar tubes that, following preparation, are allowed to harden in the upright position are designated as **agar deep tubes.** Agar deep tubes are used primarily for the study of the gaseous requirements of microorganisms. However, they may be liquefied in a boiling water bath and poured into Petri dishes, producing **agar plates,** which provide large surface areas for the isolation and study of microorganisms. The various forms of solid media are illustrated in Figure P1.2.

In addition to nutritional needs, the environmental factors must also be regulated, including proper pH, temperature, gaseous requirements, and osmotic pressure. A more detailed explanation is presented in Part 4, which deals with cultivation of microorganisms; for now, you should simply bear in mind that numerous types of media are available.



(a) Agar slants **(b)** Agar deep tube **(c)** Agar plate

Figure P1.2 **Forms of solid (agar) media**

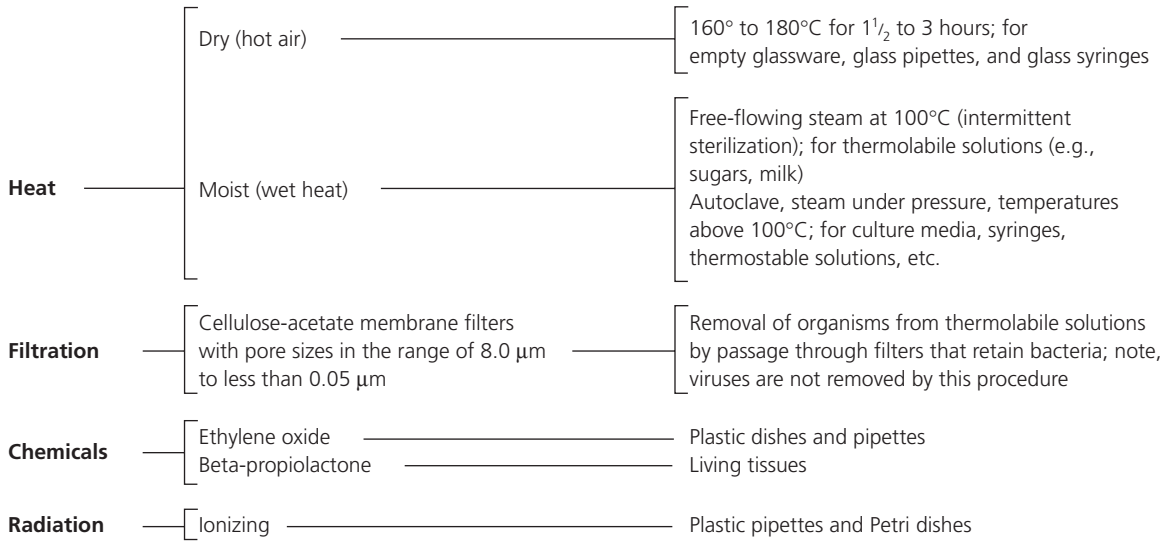


Figure P1.3 Sterilization techniques

Aseptic Technique

Sterility is the hallmark of successful work in the microbiology laboratory, and **sterilization** is the process of rendering a medium or material free of all forms of life. To achieve sterility, it is mandatory that you use sterile equipment and employ **aseptic techniques** when handling bacterial cultures. Although a more detailed discussion is presented in Part 9, which describes the control of microorganisms, **Figure P1.3** is a brief outline of the routine techniques used in the microbiology laboratory.

Culture Tubes and Petri Dishes

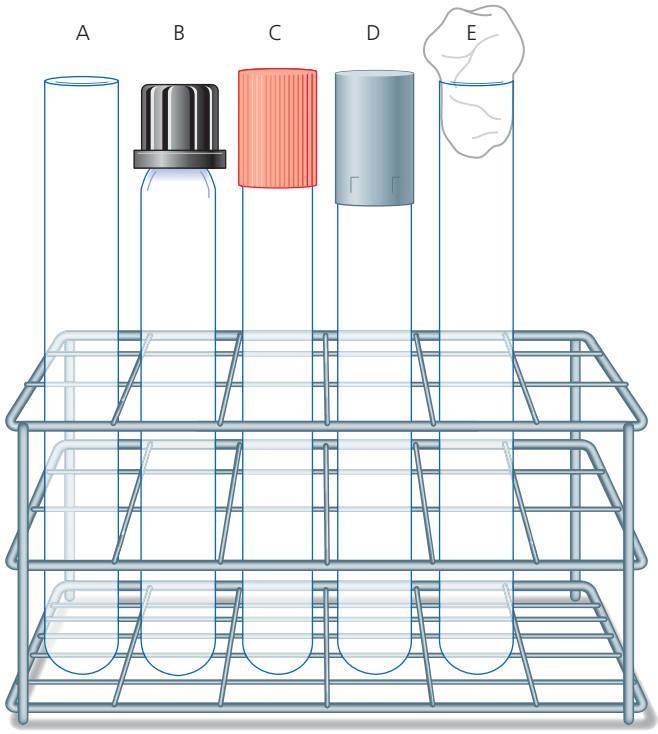
Glass **test tubes** and glass or plastic **Petri dishes** are used to cultivate microorganisms. A suitable nutrient medium in the form of broth or agar may be added to the tubes, while only a solid medium is used in Petri dishes. A sterile environment is maintained in culture tubes by various types of closures. Historically, the first type, a cotton plug, was developed by Schröeder and von Dusch in the nineteenth century. Today most laboratories use sleevelike caps (Morton closures) made of metal, such as stainless steel, or heat-resistant plastics. The advantage of these closures over the cotton plug is that they are labor-saving and, most of all, slip on and off the test tubes easily.

Petri dishes provide a larger surface area for growth and cultivation. They consist of a bottom dish portion that contains the medium and a larger top portion that serves as a loose cover. Petri dishes are manufactured in various sizes to meet different experimental requirements. For routine purposes, dishes approximately 15 cm in diameter are used. The sterile agar medium is dispensed to previously sterilized dishes from molten agar deep tubes containing 15 to 20 ml of medium, or from a molten sterile medium prepared in bulk and contained in 250-, 500-, and 1000-ml flasks, depending on the volume of medium required. When cooled to 40°C, the medium will solidify. Remember that *after inoculation, Petri dishes are incubated in an inverted position* (top down) to prevent condensation formed on the cover during solidification from dropping down onto the surface of the hardened agar. **Figure P1.4** illustrates some of the culture vessels used in the laboratory. Built-in ridges on tube closures and Petri dishes provide small gaps necessary for the exchange of air.

Transfer Instruments

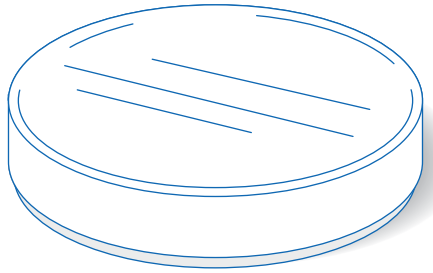
Microorganisms must be transferred from one vessel to another or from stock cultures to various media for maintenance and study. Such a transfer is called **subculturing** and must be carried out under aseptic conditions to prevent possible contamination.

Wire loops and needles are made from inert metals such as Nichrome or platinum and are inserted into metal shafts that serve as handles. They are extremely durable instruments and are easily sterilized by incineration in the blue (hottest) portion of the Bunsen burner flame.

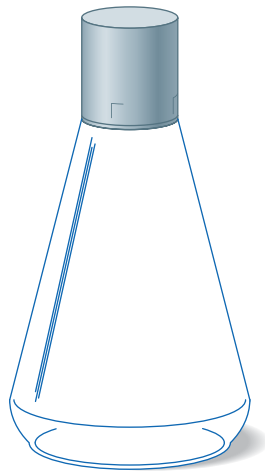


A. Bacteriological tube
B. Screw cap
C. Plastic closure
D. Metal closure
E. Nonabsorbent cotton

(a) Test tube rack with tubes showing various closures



(b) Petri dish



(c) DeLong shaker flask with closure

Figure P1.4 Culture vessels

A **pipette** is another instrument used for aseptic transfers. Pipettes are similar in function to straws; that is, they draw up liquids. They are made of glass or plastic drawn out to a tip at one end and with a mouthpiece forming the other end. They are calibrated to deliver different volumes depending on requirements. Pipettes may be sterilized in bulk inside canisters, or they may be wrapped individually in brown paper and sterilized in an autoclave or dry-heat oven.

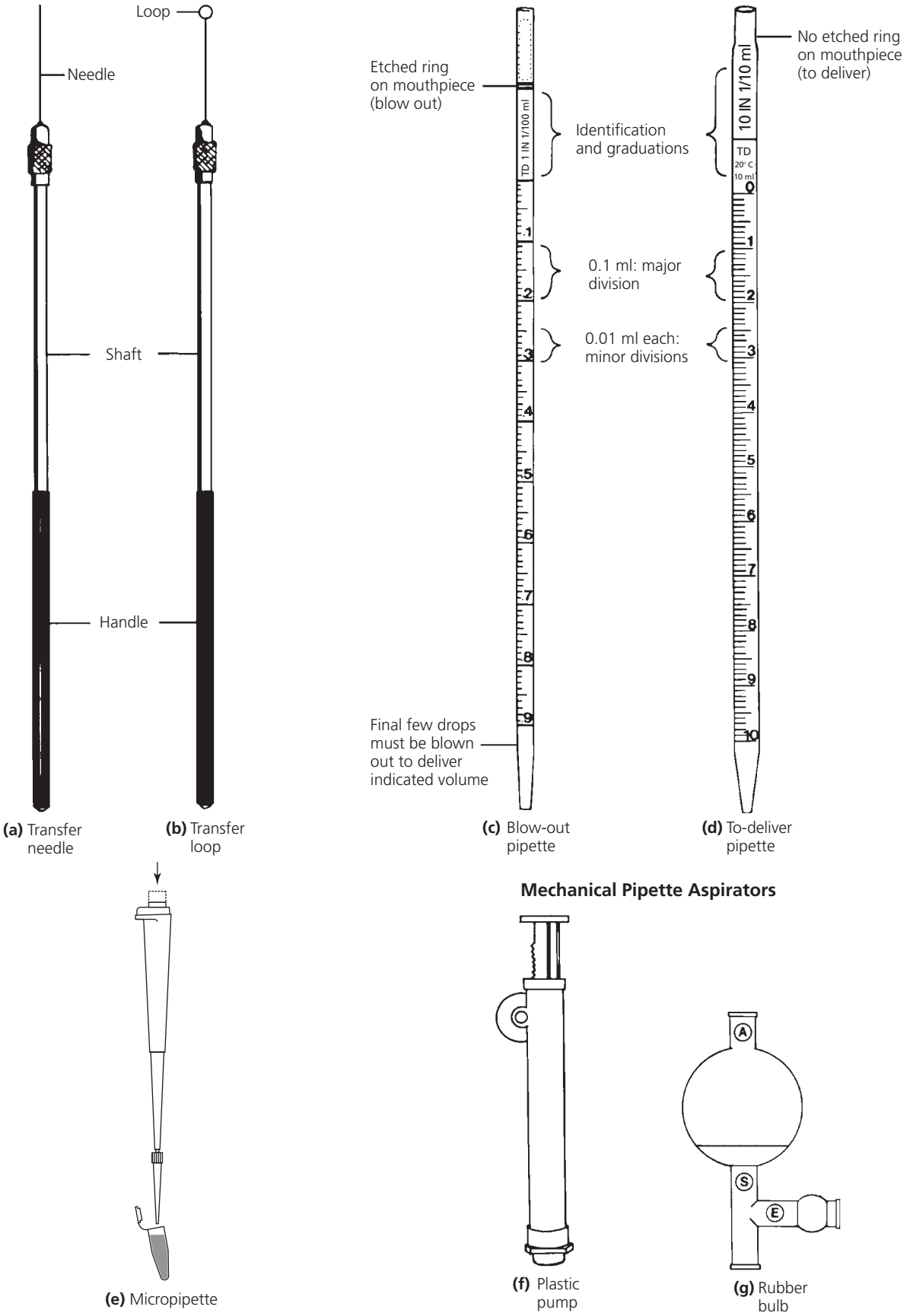
Figure P1.5 illustrates these transfer instruments. The proper procedure for the use of pipettes will be demonstrated by your instructor.

⚠ Pipetting by mouth is not permissible! Pipetting is to be performed with the aid of mechanical pipette aspirators.

Cultivation Chambers

The specific temperature requirements for growth are discussed in detail in Part 4. However, a prime requirement for the cultivation of microorganisms is that they be grown at their optimum temperature. An incubator is used to maintain optimum temperature during the necessary growth period. It resembles an oven and is thermostatically controlled so that temperature can be varied depending on the requirements of specific microorganisms. Most incubators use dry heat. Moisture is supplied by placing a beaker of water in the incubator during the growth period. A moist environment retards dehydration of the medium and thereby avoids misleading experimental results.

A thermostatically controlled **shaking waterbath** is another piece of apparatus used to



(a) Transfer needle

(b) Transfer loop

(c) Blow-out pipette

(d) To-deliver pipette

(e) Micropipette

(f) Plastic pump

(g) Rubber bulb

Figure P1.5 Transfer instruments

cultivate microorganisms. Its advantage is that it provides a rapid and uniform transfer of heat to the culture vessel, and its agitation provides increased aeration, resulting in acceleration of growth. The single disadvantage of this instrument is that it can be used only for cultivation of organisms in a broth medium.

Refrigerator

A refrigerator is used for a wide variety of purposes such as maintenance and storage of stock cultures between subculturing periods, and storage of sterile media to prevent dehydration. It is also used as a repository for thermolabile solutions, antibiotics, serums, and biochemical reagents.

Effectiveness of Hand Washing

EXPERIMENT 1

LEARNING OBJECTIVES

Once you have completed this experiment, you should understand

1. The difference between the residential flora and transient flora found on skin surfaces.
2. The effect of hand washing on the reduction of organisms on the skin.
3. The effectiveness of using soap alone or soap accompanied by surgical brushing.

Principle

Each day our hands come in contact with numerous objects and surfaces that are contaminated with microorganisms. These may include door handles, light switches, shopping carts, sinks, toilet seats, books, or even things like compost piles or body fluids, to name a few. The lack of adequate hand washing is a major vehicle in the transmission of microbial infection and disease.

The skin of a human being is sterile while *in utero* and first becomes colonized by a normal microbial flora at birth as it is passed through the birth canal. By the time you reach adulthood, your skin is calculated to contain 10^{12} (1,000,000,000,000), or one trillion, bacteria, most of which are found in the superficial layers of the epidermis and upper hair follicles. This normal flora of microorganisms is called the **resident flora,** the presence of which does not cause negative effects in healthy individuals. In fact, it forms a symbiotic relationship with your skin, which is vital to your health. This beneficial relationship can change in patients who are immunocompromised, or when residential flora accidently gains entrance to the host via inoculating needles, indwelling catheters, lacerations, and the like. Microorganisms that are less permanent and present for only short periods are termed **transient flora.** This latter flora can be removed with good hand washing techniques. The resident flora is more difficult to remove because they are found in the hair follicles and covered by hair, oil, and dead skin cells that obstruct their removal by simple hand washing with soap. Surgical scrubbing is the best means for removal of these organisms from the skin.

Surgical hand washing was introduced into medical practice in the mid-nineteenth century by the Hungarian physician Ignatz Semmelweis while working at an obstetric hospital in Vienna. He observed that the incidence of puerperal fever (child birth fever) was very high, with a death rate of about 20%. He further observed that medical students examining patients and assisting in deliveries came directly from cadaver (autopsy) laboratories without stopping to wash their hands. Upon his insistence, medical students and all medical personnel were required to wash their hands in a chloride of lime (bleach) solution before and after all patient contact. The incidence of death from puerperal fever dropped drastically to around 1%. Semmelweis's effort was responsible for the development of routine surgical scrubbing by surgeons, which has become essential practice for all surgical procedures in modern medicine.

CLINICAL APPLICATION

Preventing Nosocomial Infections

Nosocomial (hospital-acquired) infections are mainly transmitted from the unwashed hands of health care providers. Transient and residential flora on health care providers' skin can infect hospital patients whose immune systems are compromised. The cornerstone for the prevention of nosocomial infections is the meticulous hand washing and scrubbing of health care personnel. In the laboratory setting, your normal flora may contaminate patient samples and skew your result, leading to a misdiagnosis. It is important for everyone in the lab to correctly wash their hands before and after handling biological materials.

AT THE BENCH

Materials

Media

4 nutrient agar plates per student pair

Equipment

Liquid antibacterial soap, 8 sterile cotton swabs, 2 test tubes of sterile saline, Bunsen burner, glass marking pencil, surgical hand brush, Quebec colony counter, stopwatch.

Procedure Lab One

1. One student will become the washer and the other student the assistant. **The washer must not wash hands before coming to the lab.**
2. The assistant will use the glass marking pencil to label the bottoms of the nutrient agar plates. The assistant will mark two plates as "Water" and two plates as "Soap" and draw a line down the middle of each plate to divide each plate in half. For the "Water" plates, label the halves as R1, R2, R3, and R4. For the "Soap" plates, label the halves as L1, L2, L3, and L4. See Figure 1.1.
3. The assistant will aseptically dip a sterile cotton swab into the first test tube of sterile saline. To do this:
   - **a.** First light the Bunsen burner.
   - **b.** Uncap the test tube; after removing the cap, keep the cap in your hand with the inner aspect of the cap pointed away from your palm. The cap must never be placed on the laboratory bench because doing so would compromise the aseptic procedure.
   - **c.** Flame the neck of the tube by briefly passing it through the flame of the Bunsen burner.
   - **d.** Remove the tube from the flame and dip the swab in the tube, soaking it with saline. Avoid touching the sides of the tube with the swab.

   The assistant will then rub the moistened cotton swab on the pad of the washer's **right** thumb.
4. The assistant will then aseptically inoculate the half of the nutrient agar plate labeled R1 by streaking the far edge of the plate several times then making a zig zag streak only on the half labeled R1. See Figure 1.2. *Caution: Do not gouge the surface of the agar plate.*
5. The assistant will turn on the tap on the lab sink, so that the washer can wash the right hand under warm running water, **without soap,** concentrating on the thumb (rubbing the thumb over the right index and middle finger) for one minute. The assistant will turn off the tap. The washer will shake off the excess water from the hand, but not blot dry. The assistant, using a new, dry (not moistened with saline) sterile cotton swab, will obtain a sample from the right thumb pad and inoculate the section of the nutrient agar plate labeled R2 in the same way that R1 was inoculated.
6. Repeat step 5 two more times, washing the thumb for 2 minutes and then 3 minutes, respectively. The assistant will use a new, dry sterile cotton swab each time, and will aseptically inoculate R3 and R4, respectively. See Table 1.1.
7. The assistant and washer will now move to the left hand. The assistant will aseptically dip the

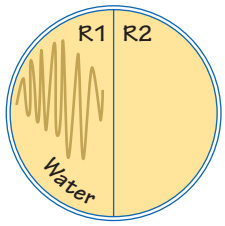


Figure 1.2 Plate inoculation

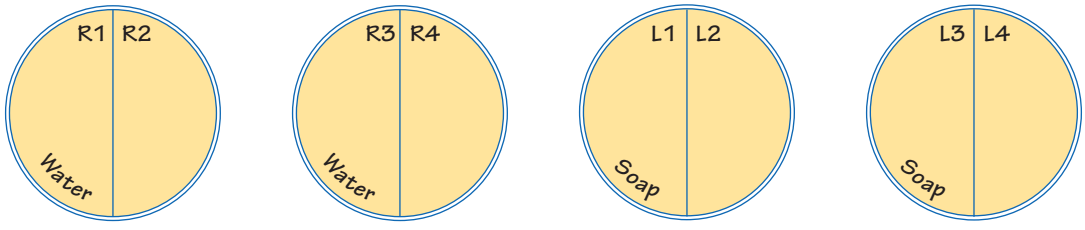


Figure 1.1 Plate labeling

TABLE 1.1 Inoculation of Nutrient Agar Plates

	WATER—RIGHT THUMB		SOAP—LEFT THUMB
R1	No wash, damp cotton swab	L1	No wash, damp cotton swab
R2	Wash 1 minute, dry cotton swab	L2	Wash with soap 1 minute, dry cotton swab
R3	Wash 2 minutes, dry cotton swab	L3	Soap and surgical brush 2 minutes, dry cotton swab
R4	Wash 3 minutes, dry cotton swab	L4	Soap and surgical brush 3 minutes, dry cotton swab

sterile cotton swab into the second test tube of sterile saline (following the process from Step 3) and will rub the moistened cotton swab over the pad of the left thumb and aseptically inoculate L1 as shown in Figure 1.2.

8. The assistant will turn on the tap of the lab's sink so that the washer can wet the thumb and index finger of the left hand under warm running water. The assistant will apply one or two drops of liquid soap to the thumb and index finger and the washer will wash for 1 minute by rubbing the thumb over the index finger. Rinse well. Shake off water from the hand but do not blot dry. The assistant will turn off the tap. The assistant will then use a dry, sterile cotton swab to obtain a sample from the washed thumb pad and inoculate L2.
9. Repeat step 8 two more times, not only using soap but also scrubbing the thumb with a surgical brush, for 2 minutes and then 3 minutes, respectively. The washer will obtain the surgical brush and the assistant will add saline to the brush to dampen it, and then add one or two drops of soap to the thumb and also the brush. *Caution: Place the brush bristles up on a dry paper towel between washings.* The assistant will use a new, dry sterile cotton swab each time, and will aseptically inoculate L3 and L4, respectively. Refer back to Table 1.1.
10. Incubate all plates in an inverted position at 37°C for 24 to 48 hours.

Procedure Lab Two

Examine and record the amount of growth found on each nutrient agar plate. Results may be determined by two methods.

1. **Macroscopically.** Visually observe the presence of growth on the surface of each agar plate in each section. Record your results in your Lab Report as 0 = no growth, 1+ = slight growth, 2+ = moderate growth, 3+ = heavy growth, and 4+ = maximum growth.
2. **Percent Growth Reduction.**
   a. Count the colonies that appear in each section of the agar plates using a Quebec colony counter. If more than 300 colonies are present, label it as "too numerous to count (TNTC)," if fewer than 30 colonies are present, label it as "too few to count (TFTC)."
   b. For sections R2, R3, R4 and L2, L3, L4, calculate the percent growth reduction from the first section, using the following equation:

$$\text{Percent reduction} = [\text{Colonies (section 1)} - \text{Colonies (section x)}] \div \text{Colonies (section 1)}$$

X = sections 2, 3, 4 for each hand