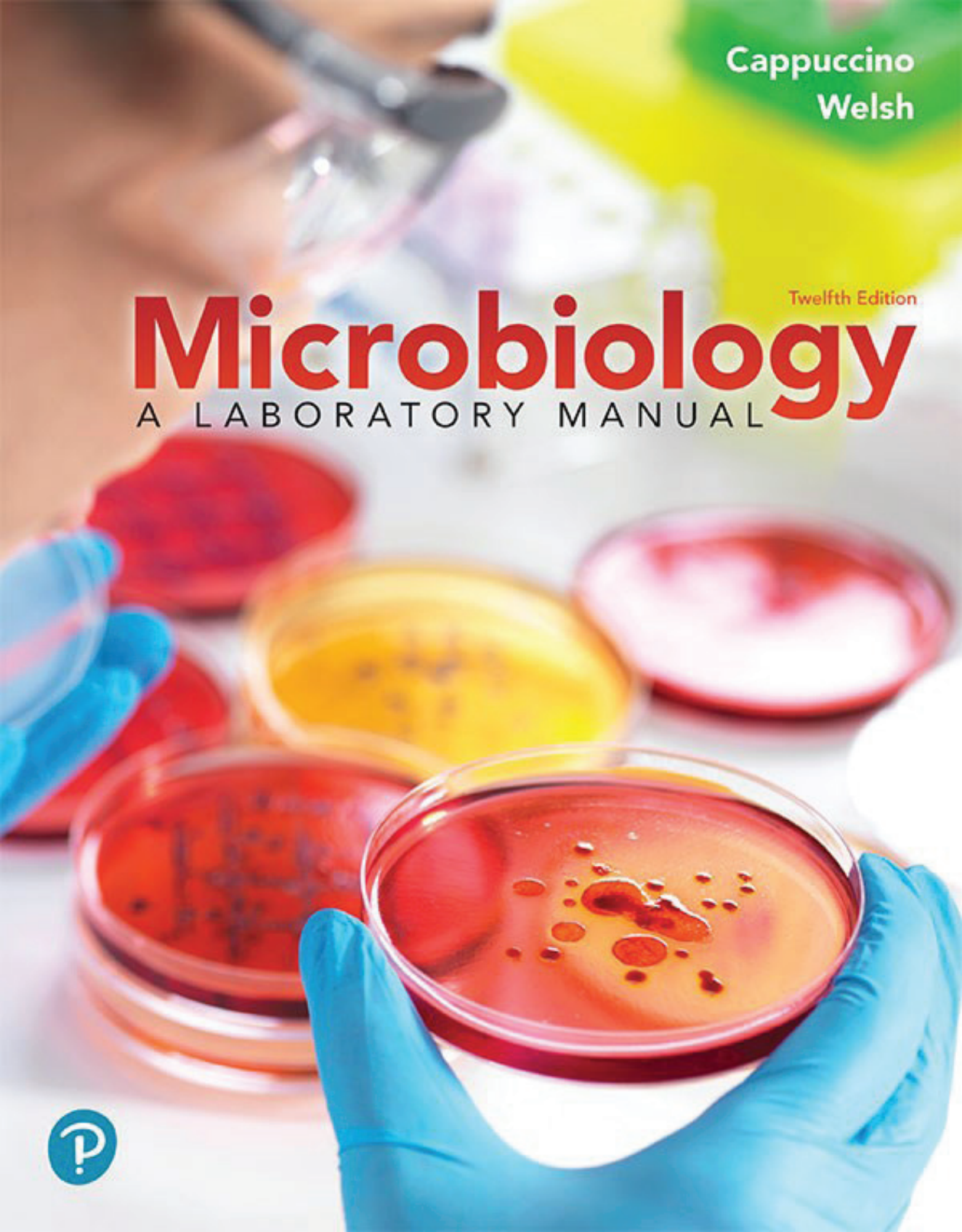
Cappuccino
Welsh

Twelfth Edition

Microbiology

A LABORATORY MANUAL



Provide New Learning Pathways to Understand the Why Behind the Science

CASE STUDY

HAND WASHING AND ASEPTIC TECHNIQUE: A CASE STUDY

You are presented with an eosin–methylene blue (EMB) agar plate that has bacterial colonies with a slight greenish, metallic sheen. Your laboratory manager explains the background for the culture you are observing on the plate: An unknown contaminate was found in a meat processing machine, and the in-house pathogen control office performed a swab and a streak on an EMB plate. After incubation and observation of the weak reaction, the manufacturers concluded that the contaminate was not *E. coli* and that no further tests were required. Upper management decided that to protect the company from potential lawsuits, they would hire the laboratory you work for to ensure that their laboratory technicians concluded correctly.

Due to cost and time restrictions, your lab is limited regarding how many assays can be performed. Using a series of biochemical tests to confirm or refute the analysis of the processing plant, you will need to determine whether the bacteria is an enteric and then whether it is an *E. coli* isolate.

Questions to Consider:

1. Does the lack of a strong reaction on the EMB plate refute the determination that the isolated bacteria are an *E. coli* culture?
2. Which series of assays would best be used to prove/disprove the *E. coli* determination?

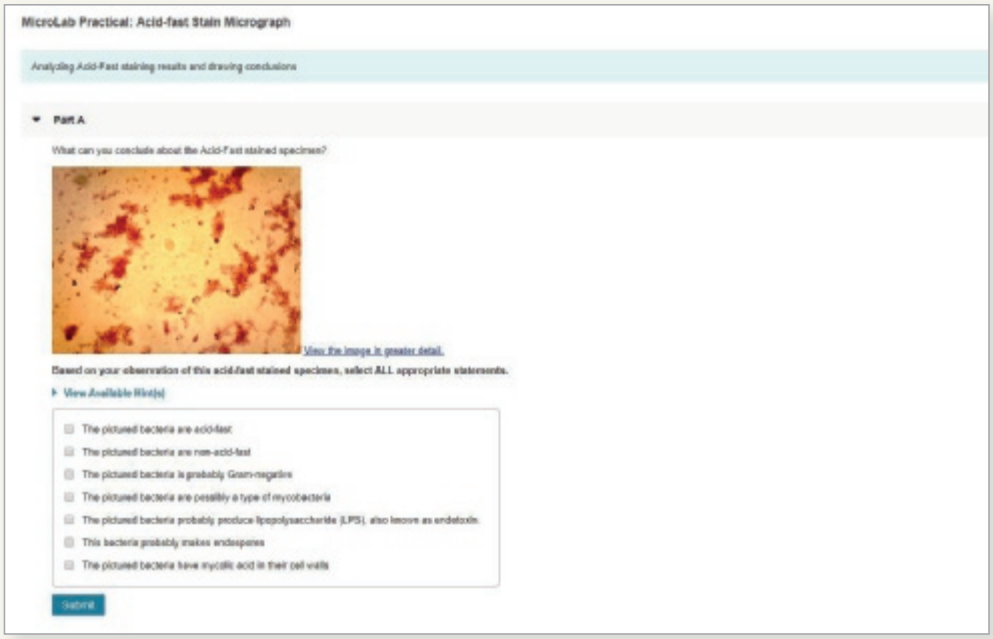
NEW! Clinical Case Studies now within each section of the lab manual bring career relevance to the lab experiments. These open-ended cases can be used to fuel class discussion and group work about the topics covered in lab.

NEW! Further Reading Sections help students know where to look in their textbook if they need more background information to understand the science behind the experiment.

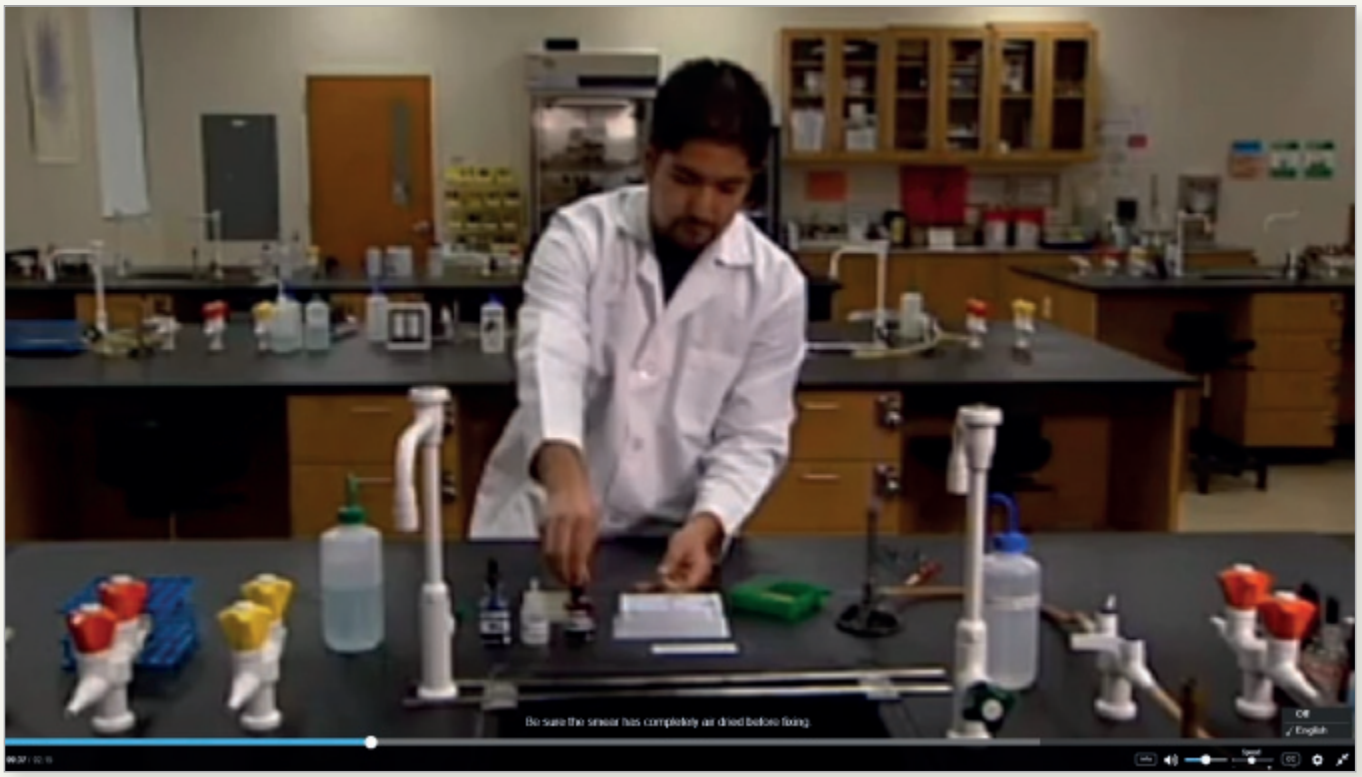
FURTHER READING

Refer to the section on antimicrobial compounds in your textbook for further information on the compounds that have an effect on bacterial cells. In your textbook's index, search under "Chemotherapy," "Antibiotics," and "Analog."

Connect Lecture and Lab with Mastering Microbiology



MicroLab Practical Activities assess students' observation skills and give them extra practice to analyze important lab tests, procedures, and results.



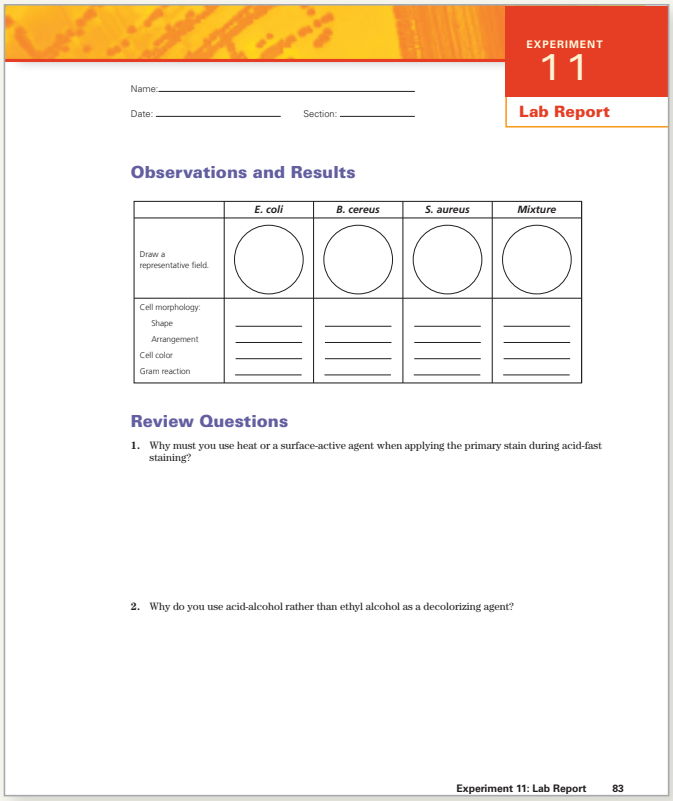
Prepare for lab with **pre-lab quizzes** for each of the 72 experiments in ***Microbiology: A Laboratory Manual*** Twelfth Edition, and then follow up to measure comprehension with **post-lab quizzes** in Mastering Microbiology™.

And Come to Class Better Prepared for Lab

Videos and Coaching Activities help instructors and students get the most out of lab time. Students can practice their lab skills virtually reviewing proper lab techniques with real-world applications. Live action video combined with molecular animation with assessment and feedback coach students how to interpret and analyze different lab results.

Lab Technique Videos give students an opportunity to see techniques performed correctly and quiz themselves on lab procedures both before and after lab time, improving confidence and proficiency. Assign as pre-lab quizzes in Mastering Microbiology and include coaching and feedback on a wide range of lab techniques.

Additional Instructor Support to Customize Your Course Your Way



EXPERIMENT
11
Lab Report

Name:_____

Date: _____ Section: _____

Observations and Results

	E. coli	*B. cereus*	*S. aureus*	*Mixture*
Draw a representative field.				
Cell morphology:				
Shape				
Arrangement				
Cell color				
Gram reaction				

Review Questions

1. Why must you use heat or a surface-active agent when applying the primary stain during acid-fast staining?

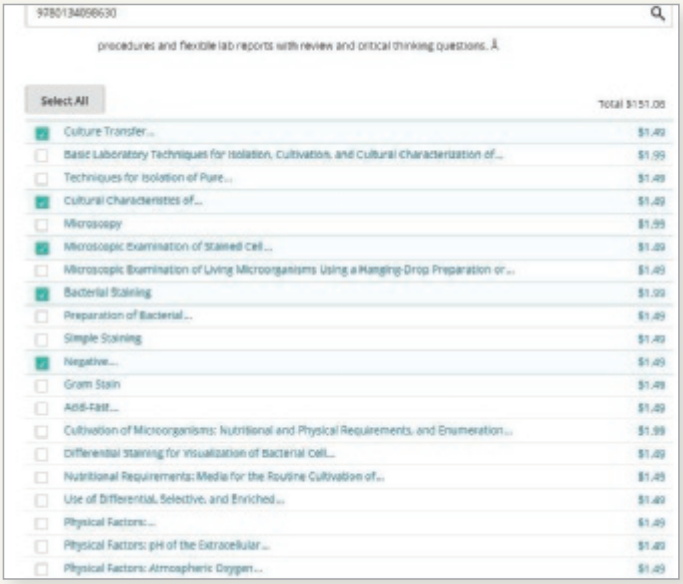
2. Why do you use acid-alcohol rather than ethyl alcohol as a decolorizing agent?

Experiment 11: Lab Report 83

Easy-to-adapt Lab Reports include blank spaces for individual course customization. Instructors can select their preferred organism.

To create the perfect lab manual, visit www.pearsoncustomlibrary.com.

Pearson Collections, www.pearsoncollections.com Your course materials should match your course, not the other way around. We offer a comprehensive catalog linked to easy-to-use curation tools. Everything is set up so you can easily design your custom content and then share it with your students.



Instructor's Guide for Microbiology: A Laboratory Manual by James G. Cappuccino, Chad T. Welsh (© 2019 0-13-520429-1 / 978-0-13-520429-0) is a valuable teaching aid for instructors. Tools include: recommended readings, detailed lists of required materials, tables for calculating the amount of media and equipment needed for your class, procedural points to emphasize, helpful tips for preparing and implementing each experiment, answers to review questions in the lab manual, and information on lab safety protocol.

Microbiology

A Laboratory Manual

Twelfth Edition

James G. Cappuccino, Emeritus
SUNY Rockland Community College

Chad Welsh
Lindenwood University

Pearson

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Contents

Preface

Microbiology is a dynamic science. It constantly evolves as more information is added to the continuum of knowledge, and as microbiological techniques are rapidly modified and refined. The twelfth 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 helps students develop 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 study life at the molecular level.

In this manual, we begin each major area of study with comprehensive introductory material, then 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 allow more time for performing the experiments. Finally, the supplies, equipment, and instrumentation for the experimental procedures can be commonly found in undergraduate institutions.

Organization

This manual consists of 72 experiments arranged into 15 parts. The experiments progress from basic and introductory, which require minimal manipulations, to more complex, which require 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 with the following components:

Learning Objectives

This introductory section defines the specific principles and/or techniques students will master.

Principle

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

Further Reading

This section aids the student in identifying the key terms and concepts within the textbook for continued reading on the topic.

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 throughout the experiment.

Materials

This comprehensive checklist 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. Appendix 6 gives a complete listing of the experimental cultures and prepared slides.

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. Appendices 4 and 5 present the chemical composition and preparation of the reagents.

Equipment Listed under this heading are the supplies and instrumentation that students need for 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 brain icon.

New to the Twelfth Edition

For this twelfth edition, the primary aim was to build upon and enrich the student experience. The changes described below impart the relevance of microbiological lab techniques to published standard protocols, and enhance student understanding in the validity of each of the microbiological procedures as they apply laboratories in both the educational and industrial setting.

Clinical Case Studies

Included with each section of the laboratory manual is a Clinical Case Study, which reviews a fictitious case that illustrates the laboratory science addressed in one or more experiments within that part. These open-ended cases have accompanying questions to facilitate class discussions about the topics covered in lab.

Further Reading

This new section, found in the introductory material for each part in the manual and within each experiment, instructs students on where to look in their textbook for more background information concerning the science behind the experiment. Worded in a general manner, this section is not specific for a singular textbook but utilizes common textbook section descriptions and the nomenclature that is found in most indexes.

Check Lists

With the lengthy lists of materials, media, and organisms required in some experiments, many students have found the preparation for the experiment daunting. To aid the students in ensuring that they have acquired all of the needed materials, these lists have been converted to check-lists.

New Experiment 36: Isolation of Fungal Species

A newly designed experiment that illustrates a method for the isolation of a singular or multiple fungal species from an environmental sample. This is a generic protocol that will allow for individual customization by Instructors to fit their labs or interests.

New Experiment 46: Detection of Enteric Bacteria on Raw Meat

Loosely based on the published protocols of the United States Department of Agriculture (USDA) and Food Safety and Inspection Service (FSIS) for the cultivation, isolation, and identification of enteric bacteria on commercially prepared meat and meat products, this laboratory experiment is based on government guidelines published in MLG 4.09.

Information Concerning Governing Bodies

Where appropriate, information concerning governing bodies, such as the USDA and its regulatory agency FSIS, has been included in the introductory material for some experiments. By drawing attention to governing bodies beyond the American Society for Microbiology (ASM) that have published laboratory standards, students are introduced to the various industry standards that regulate microbiology laboratories.

Updates and Revisions

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

- Experiment 1 Handwashing was added back to this edition, by popular demand.
- New or updated artwork in some experiments.
- Experiment 24 now has a combined laboratory procedure for the Methyl Red (MR) and Voges-Proskauer (VP) tests to minimize student
- Experiments 56 and 57 were combined into one new Experiment 57 that is now a multi-week bacterial Isolation and Transformation lab.
- Experiment 64 now also introduces the commercially available EnteroPleuri test for identifying enteric bacteria.

Instructor Resources

The Instructor Guide (ISBN 978-0-134-29869-6) is a valuable teaching aid for instructors. It was updated to reflect changes in the main text, and provides:

- Laboratory safety protocol for the instructional staff
- Laboratory safety protocol for the technical staff
- New Additional Reading research articles 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

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I would like to express my sincere condolences to the family of Dr. James Cappuccino. I appreciate their allowing the continued publication of this laboratory manual that has been his work for the past 20-plus years.

I also wish to extend my appreciation to the staff at Pearson who helped me through the creation of this manual. Specifically, I would like to thank Jennifer McGill, Coleen Morrison, Norine Strang, and Sonsy Matthews for stewarding this revision.

Chad Welsh

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.

You should observe the following basic steps 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 human immunodeficiency virus (HIV) and hepatitis B virus 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 on 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: _____

Laboratory Protocol

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. Use commercially pre-cleaned slides 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

Part 1 of this manual fully describes aseptic techniques for the transfer or isolation of microorganisms, using the necessary transfer instruments. You will acquire technical skill through repetitive practice.

Inoculating Loops and Needles: It is imperative that you incinerate the entire wire to ensure absolute sterilization. You should also briefly pass the shaft 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.

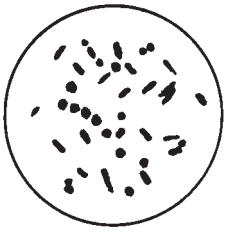
Incubation Procedure

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.

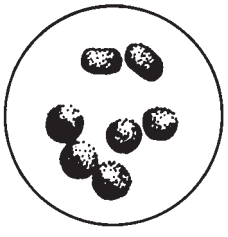
Procedure for Recording Observations and Results

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 five to ten times their actual microscopic size, as indicated by the following illustrations. For this purpose, a number two 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 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 able to

1. Identify the laboratory equipment and culture media needed to develop and maintain pure cultures.
2. Identify the types of microbial flora that live on the skin and explain how hand washing affects them.
3. Describe the concept of aseptic technique and the procedures necessary for successful subculturing of microorganisms.
4. Explain streak-plate and spread-plate isolation of microorganisms from a mixed microbial population for subsequent pure culture isolation.
5. Identify cultural and morphological characteristics of microorganisms grown in pure culture.

Introduction

Microorganisms are ubiquitous. We find them in soil, air, water, food, and sewage, and on body surfaces. In short, every area of our environment is replete with them. Microbiologists separate 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, microbiologists require basic laboratory equipment and apply 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**. All culture media are liquid, semisolid, or solid. A liquid medium lacks a solidifying agent and is called a **broth medium**. A broth medium is useful for cultivating high numbers of bacterial cells in a small volume of medium, which is particularly helpful when an assay requires a high number of healthy bacterial cells. 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, we can cultivate organisms, especially pathogens, at temperatures of 37.5°C or slightly higher without fear of the medium liquefying. A completely solid medium requires an agar concentration of 1.5% to 1.8%. A concentration of less than 1% agar results in a **semisolid medium**. A semisolid medium is useful for testing a cell's ability to grow within the agar at lower oxygen

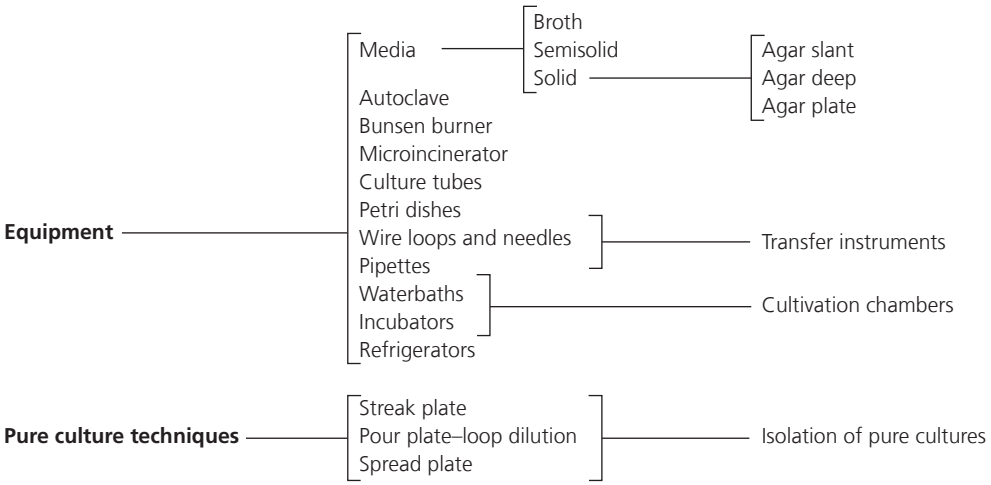
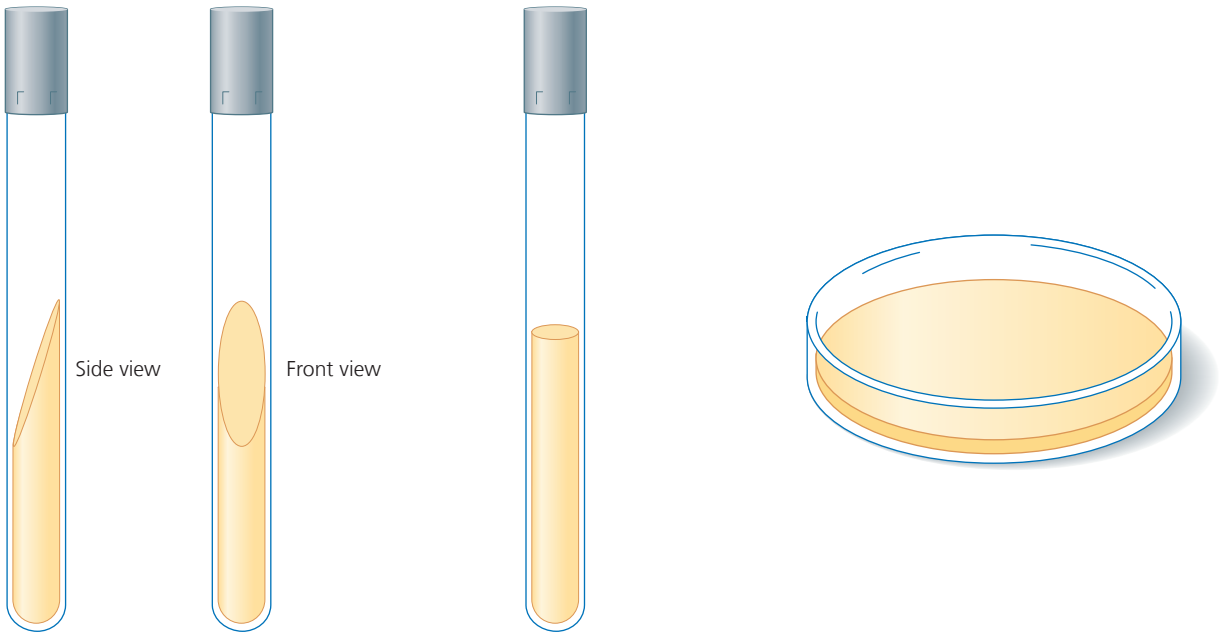


Figure P1.1 Laboratory apparatus and culture techniques

levels and for testing the species' motility. A solid medium is advantageous because 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 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, we can place solid media in test tubes, which then cool and harden in a slanted position, producing **agar slants**. These are useful for maintaining pure cultures. The slanted surface of the agar maximizes the available surface area for microorganism growth while minimizing the amount of medium required. Similar tubes that, following preparation, harden in the upright position are designated as **agar deep tubes**. Agar deep tubes are used primarily for studying gaseous requirements of microorganisms, since gas exchange between the agar at the butt of the test tube and the external environment is impeded by the height of the agar. Liquid agar medium can also be 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.



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

Figure P1.2 Forms of solid (agar) media

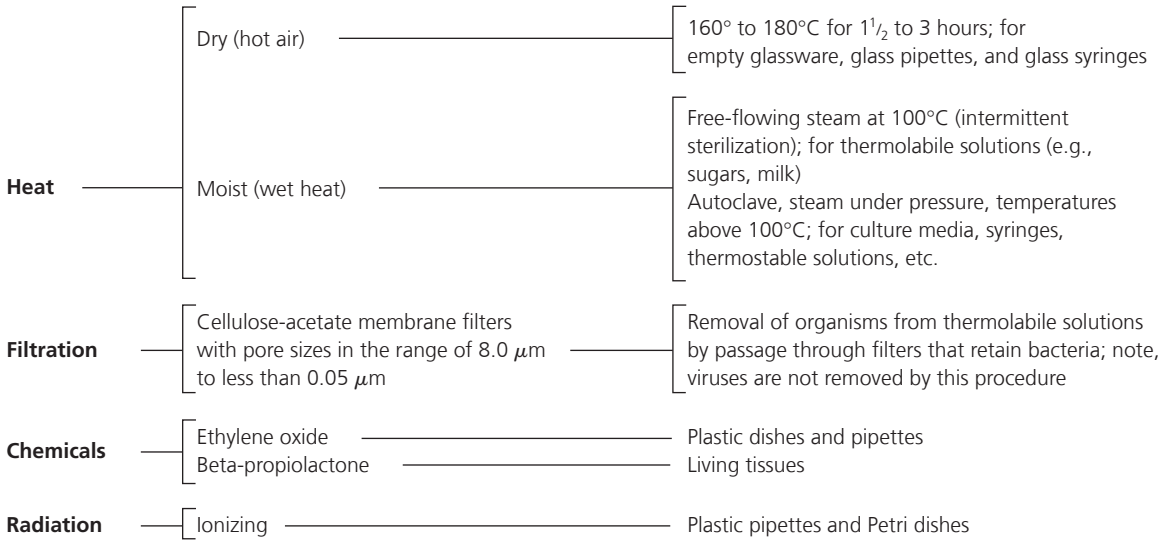


Figure P1.3 Sterilization techniques

In addition to nutritional needs, we must regulate environmental factors, including proper pH, temperature, gaseous requirements, and osmotic pressure. You can read a more detailed explanation about the cultivation of microorganisms in Part 4; for now, you should simply note that numerous types of media are available.

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. Using correct aseptic techniques minimizes the likelihood that bacterial cultures will be contaminated, and reduces the opportunity that you will be exposed to potential pathogens. Figure P1.3 is a brief outline of the routine techniques used in the microbiology laboratory, and you will learn more about the control of microorganisms in Part 9.

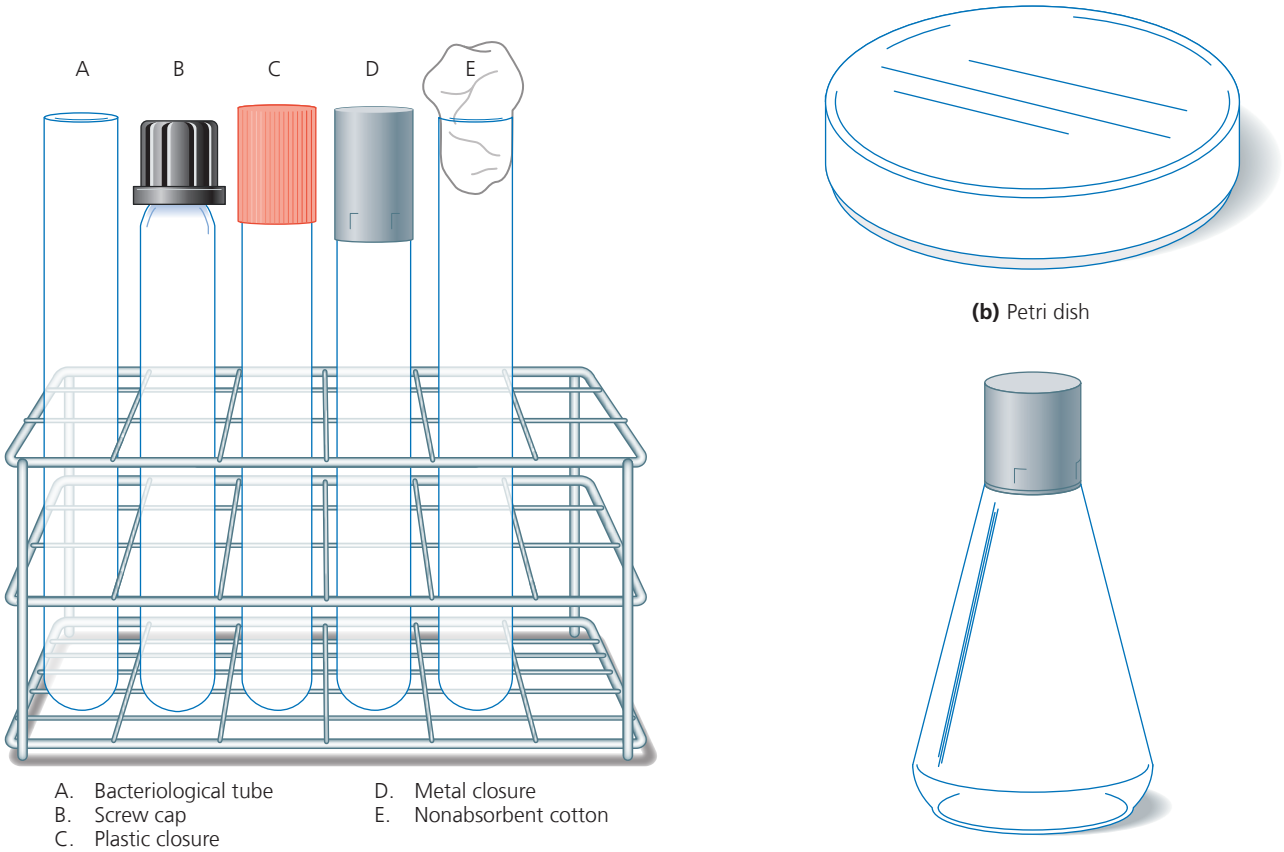
Culture Tubes and Petri Dishes

We use glass **test tubes** and glass or plastic **Petri dishes** to cultivate microorganisms. We can add a suitable nutrient medium in the form of broth or agar to the tubes, while we use only a solid medium in Petri dishes. We maintain a sterile environment in culture tubes by various types of closures. Historically, the first type, a cotton plug, was developed by Heinrich G. F Schröeder and Theodor von Dusch in the nineteenth century. Today most laboratories use sleeve-like 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, that they 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, we use dishes approximately 15 cm in diameter. The sterile agar medium is dispensed to previously sterilized dishes from molten agar deep tubes containing 15 ml 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. For this reason, we should label Petri dishes on the bottom of the dish. This makes it easier to read the label and minimizes confusion if two Petri dish covers are interchanged. 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. This transfer



(a) Test tube rack with tubes showing various closures

(b) Petri dish

(c) DeLong shaker flask with closure

Figure P1.4 Culture vessels

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 wire loop is useful for transferring a small volume of bacteria onto the surface of an agar plate or slant. We use a needle to inoculate a culture into a broth medium or into an agar deep tube.

A **pipette** is another instrument used for aseptic transfers. Pipettes are similar in function to straws; that is, they draw up liquids. They are glass or plastic, and drawn out to a tip at one end, 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. A micropipette (commonly called a "pipetter") with a disposable, single-use plastic tip is useful for transferring small volumes of liquid (less than 1 ml).

Figure P1.5 illustrates these transfer instruments. Your instructor will demonstrate the proper procedure for using pipettes.

⚠ Pipetting by mouth is not permissible! Pipetting must be performed with mechanical pipette aspirators.

Cultivation Chambers

Part 4 discusses specific temperature requirements for growth; however, a prime requirement for the cultivation of microorganisms is that they be grown at their optimum temperature. We use an **incubator** to maintain optimum temperature during the necessary growth period. It resembles an oven, and is thermostatically controlled so

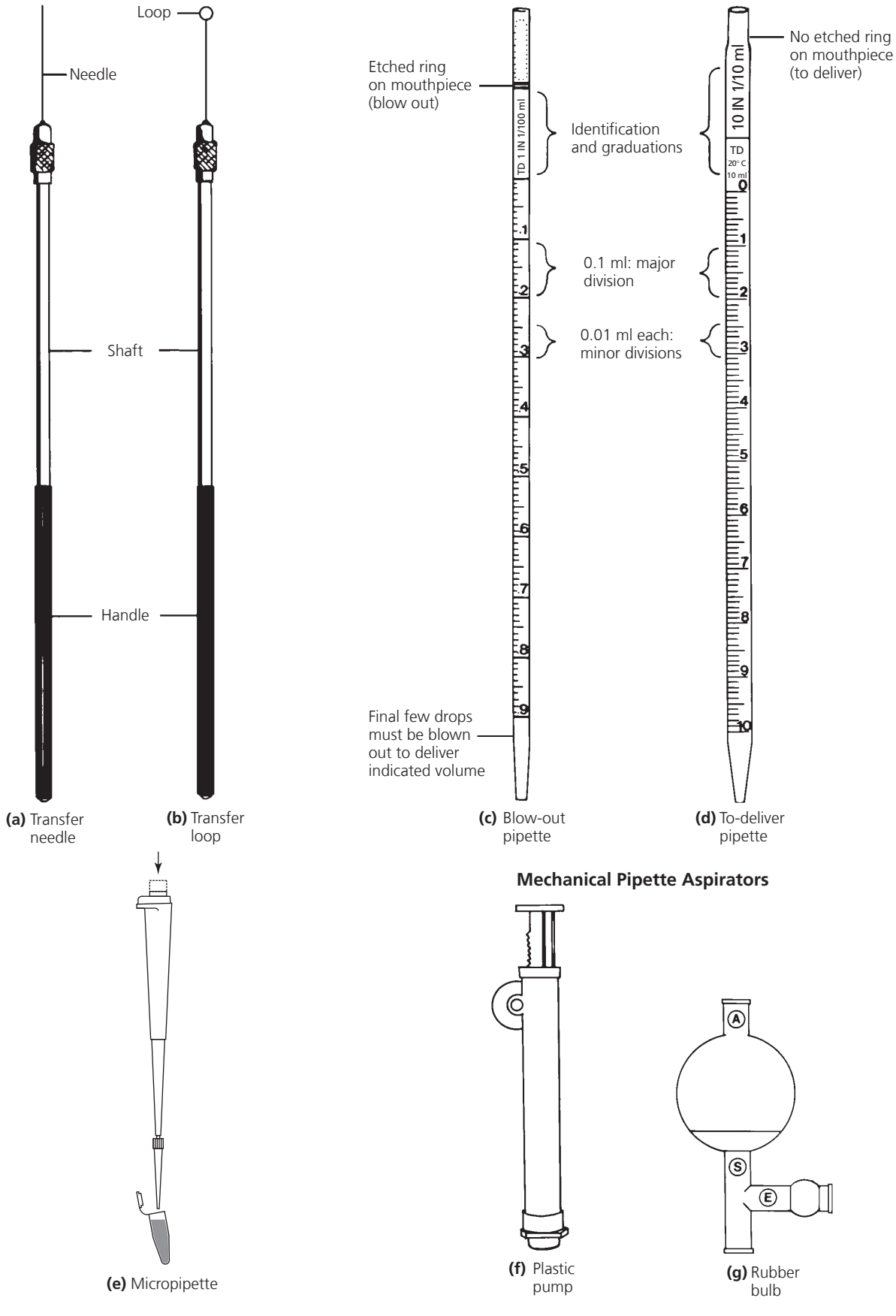


Figure P1.5 **Transfer instruments**

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 helps avoids misleading experimental results.

A thermostatically controlled **shaking waterbath** is another piece of apparatus used to 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 primary disadvantage of this instrument is that it can be used only for cultivation of organisms in a broth medium.

Many laboratories also use shaking incubators that utilize dry air incubation to promote aeration of the broth medium. This method has a distinct advantage over a shaking waterbath, since there is no chance of cross contamination from microorganisms that might grow in the waterbath.

Refrigerator

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

FURTHER READING

Refer to the section on microbial growth in your textbook for more information on materials and techniques utilized in the cultivation of bacteria. Search the index for the specific terms "Agar," "Colony," and "Sterile."

CASE STUDY

HAND WASHING AND ASEPTIC TECHNIQUE

A local microbiological testing laboratory service, Aureus Systems, notified its regional headquarters about a possible contamination issue in either its Quality Assurance/Quality Control (QA/QC) lab or in its testing center proper. As an outside adviser, you have been hired to investigate the situation and to monitor the laboratory procedures of this local branch. Upon your arrival, a senior lab technician (John Doe) allows you to shadow him and answers your questions for the week of your visit. During your week, you notice some instances of gross indifference to standard laboratory practices concerning personal hygiene and personal protection practices.

On numerous instances you have recorded Mr. Doe removing his latex gloves and continuing to handle specimens and laboratory media without washing his hands. Many times, Mr. Doe has been reprimanded for this practice, as well as for failure to wash his hands before leaving the lab room itself. Mr. Doe argues that his aseptic technique practices are at a high enough standard that he is incapable of contaminating any specimens that he is working on in the lab. On numerous occasions his supervisors have recorded that stock media preparations used by Mr. Doe and other laboratory technicians have been contaminated with unknown microbes.

The regional headquarters requires laboratory proof that Mr. Doe—and not the equipment or the lab environment—is the source of the contamination.

Questions to Consider:

1. Why is it important to wash your hands BEFORE and AFTER using bacterial cultures?
2. How would you isolate the contaminating microbes from the contaminated stocks to determine what species they are?
3. Why would the use of "aseptic technique" be important in a testing lab, or any microbiology lab?

EXPERIMENT 1

Effectiveness of Hand Washing

LEARNING OBJECTIVES

Once you have completed this experiment, you should be able to:

1. Differentiate between the residential flora and transient flora found on skin surfaces
2. Determine the effect of hand washing on the reduction of organisms on the skin
3. Explain 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.

Our skin 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 accidentally gains entrance to the host via inoculating needles, indwelling catheters, lacerations, and the like. Microorganisms that are less permanent, present for only short periods, are termed **transient flora**. This latter flora can be removed with good hand washing techniques. Resident flora is more difficult to remove because it is found in the hair follicles and is covered by hair, oil, and dead skin cells that obstruct its 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 Ignaz Semmelweis while working at an obstetric hospital in Vienna. He observed that the incidence of puerperal fever (childbirth 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 led to the development of routine surgical scrubbing by surgeons, which has become essential practice for all surgical procedures in modern medicine.

FURTHER READING

Refer to the sections on hand washing and laboratory hygiene to review proper laboratory protocols and microbe handling safety. In your textbook's index, search under the terms "Hygiene" and "Aseptic Technique."

CLINICAL APPLICATION

Preventing Nosocomial Infections

Nosocomial (hospital-acquired) infections are mainly transmitted from the unwashed hands of healthcare providers. Transient and residential flora on healthcare providers' skin can infect hospital patients whose immune systems are compromised. The cornerstone for the prevention of nosocomial infections is meticulous hand washing and scrubbing by healthcare personnel. In the laboratory setting, your normal flora may contaminate patient samples and skew your results, 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
- ❑ Microincinerator
- ❑ Glass marking pencil
- ❑ Surgical hand brush
- ❑ Quebec colony counter
- ❑ Stopwatch

Procedure Lab One

1. One student becomes the washer and the other student the assistant. **The washer must not wash hands before coming to the lab**.
2. The assistant uses the glass marking pencil to label the bottoms of the nutrient agar plates. The assistant marks two plates as "Water" and two plates as "Soap," and draws 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 aseptically dips a sterile cotton swab into the first test tube of sterile saline. To do this, complete the following steps.
   - **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 then rubs the moistened cotton swab on the pad of the washer's **right** thumb.
4. The assistant then aseptically inoculates the half of the nutrient agar plate labeled R1 by streaking the far edge of the plate several times, then making a zigzag streak on only the half labeled R1. See Figure 1.2. *Caution: Do not gouge the surface of the agar plate.*
5. The assistant turns 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 turns off the tap. The washer shakes off the excess water from the hand, but does not blot dry. The assistant, using a new dry (not moistened with saline) sterile cotton swab, obtains a sample from the right thumb pad and inoculates the section of

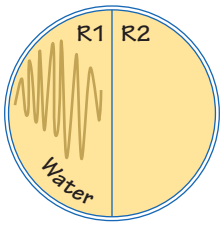


Figure 1.2 Plate inoculation.

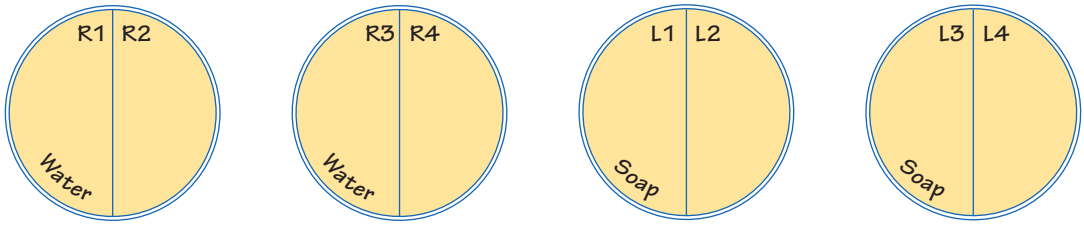


Figure 1.1 Plate labeling

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 uses a new dry sterile cotton swab each time, and aseptically inoculates R3 and R4, respectively. See **Table 1.1**.
7. The assistant and washer now move to the left hand. The assistant aseptically dips the sterile cotton swab into the second test tube of sterile saline (following the process from step 3), rubs the moistened cotton swab over the pad of the left thumb, and aseptically inoculates L1 as shown in Figure 1.2.
8. The assistant turns 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 applies one or two drops of liquid soap to the thumb and index finger and the washer washes 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 turns off the tap. The assistant then uses a dry sterile cotton swab to obtain a sample from the washed thumb pad and inoculates L2.

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

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 holds the surgical brush and the assistant adds saline to the brush to dampen it, and then adds one or two drops of soap to the thumb and also to the brush. *Caution: Place the brush bristles-up on a dry paper towel between washings.* The assistant uses a new dry sterile cotton swab each time, and aseptically inoculates 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, L2, L3, and L4, calculate the percent growth reduction from the first section, using the following equation:

Percent reduction = [Colonies (section 1) −Colonies (section x)] ÷ Colonies (section 1)

X = sections 2, 3, 4 for each hand