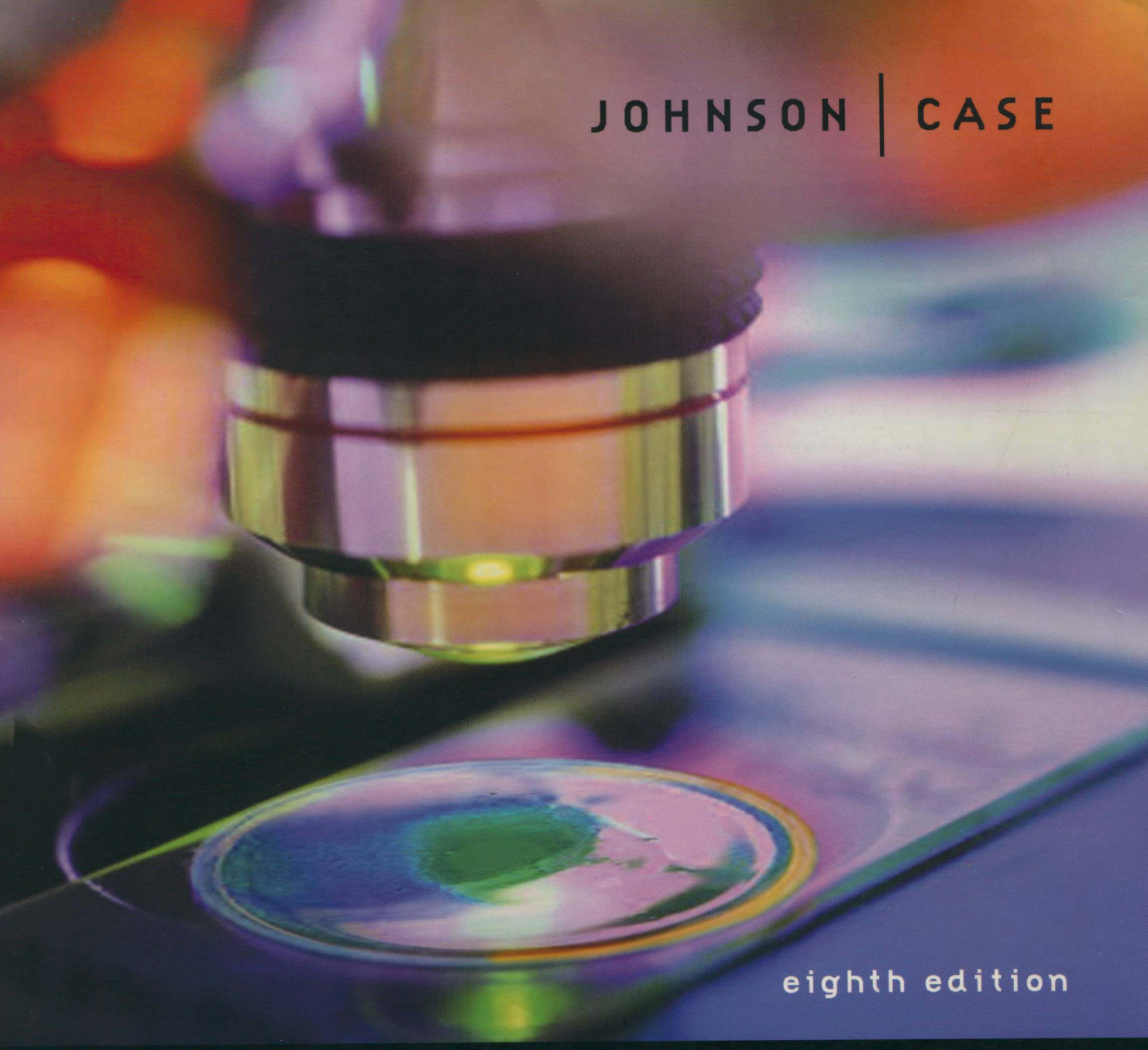

JOHNSON | CASE

eighth edition

LABORATORY EXPERIMENTS IN MICROBIOLOGY

Laboratory Experiments in Microbiology

EIGHTH EDITION

Ted R. Johnson
St. Olaf College

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GENERAL SAFETY IN THE LABORATORY

During your microbiology course, you will learn how to safely handle fluids containing microorganisms. Through practice you will be able to perform experiments so that bacteria, fungai, and viruses remain in the desired containers, uncontaminated by microbes in the environment. These techniques, called **aseptic techniques,** will be a vital part of your work if you are going into health care or biotechnology.

1. Do not eat, drink, smoke, store food, or apply cosmetics in the laboratory.
2. Wear shoes at all times in the laboratory.
3. Tie back long hair.
4. Disinfect work surfaces at the beginning and end of every lab period and after every spill.
5. Wash your hands before and after every laboratory period. Because bar soaps may become contaminated, use liquid or powdered soaps.
6. Use mechanical pipetting devices; do not use mouth pipetting.
7. Place a disinfectant-soaked paper towel on the desk while pipetting.
8. Wash your hands immediately and thoroughly if they become contaminated with microorganisms.
9. Cover spilled microbial cultures with paper towels, and saturate the paper towels with disinfectant. Leave covered for 20 minutes, and then clean up the spill and dispose of the towels.
10. Do not touch broken glassware with your hands; use a broom and dustpan. Place broken glassware contaminated with microbial cultures or body fluids in the To Be Autoclaved container. (See p. xiv for what to do with broken glassware that is not contaminated.)
11. Place glassware and slides contaminated with blood, urine, and other body fluids in disinfectant.
12. Work only with your own body fluids and wastes in exercises requiring saliva, urine, blood, or feces, to prevent transmission of disease. The Centers for Disease Control and Prevention (CDC) state that "epidemiologic evidence has implicated only blood, semen, vaginal secretions, and breast milk in transmission of HIV" (*Biosafety in Microbiological and Biomedical Laboratories*, www.cdc.gov).
13. Don't perform unauthorized experiments.
14. Don't use equipment without instruction.
15. Don't engage in horseplay in the laboratory.
16. Enjoy lab and make a new friend.

Procedures marked with this biohazard icon should be performed carefully to minimize the risk of transmitting disease.

Preface

Laboratory Experiments in Microbiology, Eighth Edition, is designed to supplement any nonmajors microbiology textbook. Co-authored by Christine L. Case, this manual is an ideal companion to *Microbiology: An Introduction*, Ninth Edition, by Tortora, Funke, and Case. Our goal is to provide a manual of basic microbiological techniques with applications for undergraduate students in diverse areas, including the biological sciences, the allied health sciences, agriculture, environmental science, nutrition, pharmacy, and various preprofessional programs. This manual contains 57 thoroughly class-tested exercises covering every area of microbiology. Most exercises require about 1½ hours of laboratory time. By selecting an appropriate combination of exercises, the instructor can provide learning experiences to meet the needs of a particular course.

Our Approach

Our goal in writing this manual has been twofold—to teach microbiological techniques and to show students the importance of microbes in our daily lives and their central roles in nature. *All* of the laboratory techniques, thinking skills, and safety procedures recommended by the American Society for Microbiology are included in this manual.

Laboratory safety is our primary concern. Many students are preparing for work in a clinical environment and need to learn to handle biologically contaminated materials. Students preparing for work in biotechnology and other laboratories must master aseptic techniques to avoid contamination of their samples. Students must not only learn but also practice these safety techniques so that safety becomes part of their professional behavior. We have followed the Centers for Disease Control and Prevention guidelines for the safe handling of microbes and human body fluids. Students are instructed to work only with their own body fluids. We have included a safety contract that students can hand in to their instructors to indicate that they understand safety requirements. See the section entitled Specific Hazards on page xii and the following sections of the Introduction for specific safety suggestions. At key points in the exercises, safety boxes appear. These are marked with either a biohazard logo ☣ or a general safety logo ⚠ indicating appropriate safety techniques.

Almost every exercise includes an actual experiment requiring students to analyze data. We hope in this way to promote *analytical reasoning* and to make laboratory sessions interesting for students as well as to provide a variety of opportunities for *reinforcement* of the technical skills they have learned. Each exercise has a series of Critical Thinking questions to enhance students' investigative skills. To further demonstrate some practical uses of microbiology, we have frequently included material with direct application to procedures performed in clinical and commercial laboratories.

Scope and Sequence

This manual is divided into 13 parts. The introduction to each part explains the unifying theme for that part. Exercises in the first three parts provide sequential development of fundamental techniques. The remaining exercises are as independent as possible to allow the instructor to select the most desirable sequence. A Techniques Required section preceding each experiment lists prerequisites from earlier exercises.

Part One, Microscopy, emphasizes observation through the microscope. Practice in use and care of the microscope is followed by observations of microbes, to familiarize students with their sizes. Phase-contrast and brightfield microscopy are used to examine living material.

Part Two, Staining Methods, begins with an explanation of how to handle bacterial cultures and teaches the use of the most common stains, including preparation of stained samples and their examination. A highly reliable capsule stain is included. Exercise 8, Morphologic Unknown, introduces the concept of unknowns and can be used to test knowledge of staining methods.

Part Three, Cultivation of Bacteria, stresses aseptic technique and covers the isolation of bacterial strains and the maintenance of bacterial cultures. An exercise dealing with special media prepares the student for the next part.

Part Four, Microbial Metabolism, includes five exercises on bacterial metabolism that provide the tools for Exercises 18, 33, and 51, consisting of unknown identifications. These exercises are especially useful for learning principles of metabolism. Exercise 18, Rapid Identification Methods, demonstrates numerical identification.

Part Five, Microbial Growth, deals with the effects of environmental conditions, such as temperature and the presence of oxygen, on growth. Biofilms are grown and observed in Exercise 21.

Part Six, Control of Microbial Growth, provides practical applications of concepts of microbial growth. Methods of controlling unwanted microbes in food or in a clinical environment are examined in this part.

Part Seven, Microbial Genetics, is of particular interest because of recent notable advances in this field. Exercise 27 is a unique experiment in which students look at expression in two operons using green fluorescent protein. Exercise 28 demonstrates the isolation of bacterial mutants. Exercise 29 allows students to isolate DNA and transform cells without special equipment. DNA fingerprinting is introduced in Exercise 30. Transformation by an antibiotic resistance plasmid is performed in Exercise 31. Restriction enzyme digestion and agarose gel electrophoresis are used to analyze the plasmid. In Exercise 32, suspected chemical carcinogens are tested by the Ames test.

Part Eight, The Microbial World, examines the diversity of microorganisms. Information and techniques learned in previous exercises are used to identify a bacterial unknown in Exercise 33. Cyanobacteria are included in Exercise 36, Phototrophs: Algae and Cyanobacteria. The morphology and ecological niches of free-living eukaryotic organisms studied in microbiology are examined in Exercises 34 through 37.

Part Nine, Viruses, provides an opportunity to isolate, cultivate, and quantify bacteriophages. Students determine the host range of a plant virus in Exercise 39.

Part Ten, Interaction of Microbe and Host, introduces basic concepts of epidemiology. Methods of tracking and identifying causes of infectious diseases are practiced in the exercises in this part.

Part Eleven, Immunology, covers the host's response to infectious disease with exercises on innate immunity and adaptive immunity (agglutination tests and the ELISA technique).

Part Twelve, Microorganisms and Disease, emphasizes procedures employed in the clinical laboratory. In Exercise 51, students identify an unknown from a simulated clinical sample.

Part Thirteen, Microbiology and the Environment, includes standard methods for the examination of food. The MUG test is used to examine water for bacteriological quality. Exercise 55, Microbes Used in the Production of Foods, offers an opportunity to study food microbiology. Exercise 56, Microbes in Soil: The Nitrogen and Sulfur Cycles, provides an example of biogeochemical cycles and soil microbiology. Exercise 57, Microbes in Soil: Bioremediation, is a unique exercise illustrating the use of bacteria for cleaning up environmental pollution.

The appendices at the end of the manual provide a convenient reference to techniques required in several exercises. Appendix H includes six identification keys to bacteria.

Organization of Each Exercise

The exercises in this manual may involve mastering a skill or procedure or understanding a particular concept. Most of the exercises are investigative by design, and the student is asked to analyze the experimental results and draw conclusions. The exercises are organized as follows:

- Each exercise begins with a section called *Objectives*, which lists skills or concepts to be mastered in that exercise. The objectives can be used to test mastery of the new material after completing the exercise.
- The *Background* provides definitions and explanations for each exercise. The student is expected to refer to a textbook for more detailed explanations of the concepts introduced in the laboratory exercises.
- *Materials* lists include supplies, media, and equipment needed for the exercise.
- *Cultures* lists identify the living organisms required for the exercise.
- *Techniques Required* gives a list of techniques needed to complete each exercise.
- *Procedure* then provides step-by-step instructions, stated as simply as possible and frequently supplemented with diagrams. Questions are occasionally asked in the Procedure section to remind the student of the rationale for a step.
- The *Laboratory Report* is designed to help students learn to collect and present data in a systematic fashion. Students are asked to write the *Purpose* of the exercise so they can relate the laboratory activity to their learning. Where appropriate, exercises ask students to formulate their *Hypotheses*. Tables are provided to record *Data* or *Results*. Occasionally, students are asked to write their *Expected Results* using information provided in the Background and their own experience. Students may be asked to write their *Conclusions* following the Data or Results. Usually, *Questions* in the Laboratory Report ask for interpretation of results. The Questions are designed to lead the student from a collection of data or observations to a conclusion. In most instances, the results for each student team will be unique; they can be compared with the information given in the Background and other references but will not be identical to those references. The range of questions in each exercise requires students to think about their results, recall facts, and then use this information to answer the questions. *Critical Thinking* questions are designed to help students use their new knowledge and practice analytic skills.

Illustrations

This book is generously illustrated with diagrams of procedures and 16 pages of photographs in full color. This section has been expanded and updated in this edition to include larger and more-detailed photos. There are two photo quizzes in the color section in which students identify an unknown.

For additional photographs of lab results, students can visit the laboratory supplement on the Microbiology Place web site (http://www.microbiologyplace.com). Photographs of media and techniques help students interpret their results without giving answers and actual lab results.

Preparation Guide

The comprehensive *Preparation Guide* (ISBN 0-8053-8293-3) provides the instructor with all the information needed to set up and teach a laboratory course with this manual. It includes the following:

- General instructions for setting up the lab.
- Information on obtaining and preparing cultures, media, and reagents.
- A master table showing the techniques required for each exercise.
- Cross-references for each exercise to specific pages in *Microbiology: An Introduction*, Ninth Edition.
- For each exercise: helpful suggestions, detailed lists of materials needed, and answers to all the questions in the student manual.

To make *Laboratory Experiments in Microbiology*, Eighth Edition, easy to use, we have carefully designed the experiments to use inexpensive, readily available, nonhazardous materials. Moreover, the exercises have been thoroughly tested in our classes in Minnesota and California by students with a wide variety of talents and interests. Our students have enjoyed their microbiology laboratory experiences; we hope yours will, too!

Acknowledgments

We are most grateful to the following individuals for their time, talent, and interest in our work. Each person carefully read and edited critical parts of the manuscript.

Chuck Hoover of the University of California, San Francisco, for making us aware of the new techniques used in dental microbiology for Exercise 48.

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Anne Jayne of the University of San Francisco for offering suggestions and a capsule stain.

Patricia Carter of Skyline College for her invaluable assistance in preparing materials and proofreading.

We are indebted to St. Olaf College and Skyline College for providing the facilities and resources in which innovative laboratory exercises can be developed.

We would like to commend the staff at Benjamin Cummings for their support. In particular, we thank Leslie Berriman, our Executive Editor; Blythe Robbins, for her editorial skill and meticulous care; and David Novak, for expertly guiding this manual through the production process.

Last, but not least, our gratitude goes to Michelle Johnson, who gave her professional insights and was a sustaining presence, and Don Biederman, who provided timely encouragement and support.

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A Special Note to Students

This book is for you. The study of microbiology is dynamic because of the diversity of microbes and the variability inherent in every living organism. Outside of the laboratory—on a forest walk or tasting a fine cheese—we experience the activities of microbes. We want to share our excitement for studying these small organisms. Enjoy!

TED R. JOHNSON
CHRISTINE L. CASE

Introduction

Life would not long remain possible in the absence of microbes.

LOUIS PASTEUR

Welcome to microbiology! Microorganisms are all around us, and as Pasteur pointed out over a century ago, they play vital roles in the ecology of life on Earth. In addition, some microorganisms provide important commercial benefits through their use in the production of chemicals (including antibiotics) and certain foods. Microorganisms are also major tools in basic research in the biological sciences. Finally, as we all know, some microorganisms cause disease—in humans, other animals, and plants.

In this course, you will have firsthand experience with a variety of microorganisms. You will learn the techniques required to identify, study, and work with them. Before getting started, you will find it helpful to read through the suggestions on the next few pages.

Suggestions to Help You Begin

1. Science has a vocabulary of its own. New terms will be introduced in **boldface** throughout this manual. To develop a working vocabulary, make a list of these new terms and their definitions.
2. Because microbes are not visible without a microscope, common names have not been given to them. The word *microbe*, now in common use, was introduced in 1878 by Charles Sedillot. The microbes used in the exercises in this manual are referred to by their *scientific names*. The names will be unfamiliar at first, but do not let that deter you. Practice saying them aloud. Most scientific names are taken from Latin and Greek roots. If you become familiar with these roots, the names will be easier to remember.
3. Microbiology usually provides the first opportunity undergraduate students have to experiment with *living organisms*. Microbes are relatively easy to grow and lend themselves to experimentation. Because there is variability in any population of living organisms, not all the experiments will "work" as the lab manual says. The following exercise will illustrate what we mean:

   Write a description of *Homo sapiens* for a visitor from another planet: _____

   _____

   _____

   _____

   _____

   _____

   _____

   After you have finished, look around you. Do all your classmates fit the description exactly? Probably not. Moreover, the more detailed you make your description, the less conformity you will observe. During lab, you will make a detailed description of an organism and probably find that this description does not match your reference exactly.
4. Microorganisms must be cultured or grown to complete most of the exercises in this manual. Cultures will be set up during one laboratory period and will be examined for growth in the next laboratory period. Accurate record keeping is therefore essential. Mark the steps in each exercise with a bright color or a bookmark so you can return to complete your Laboratory Report on that exercise. *Accurate records* and *good organization* of laboratory work will enhance your enjoyment and facilitate your learning.
5. *Observing* and *recording* your results carefully are the most important parts of each exercise. Ask yourself the following questions for each experiment:

   What did the results indicate?

   Are they what I expected? If not, what happened?
6. If you do not master a technique, try it again. In most instances, you will need to use the technique again later in the course.
7. Be sure you can answer the questions that are asked in the Procedure for each exercise. These questions are included to reinforce important points that will ensure a successful experiment.
8. Finally, carefully study the general procedures and safety precautions that follow.

General Procedures in Microbiology

In many ways, working in a microbiology laboratory is like working in the kitchen. As some famous chefs have said:

> *Our years of teaching cookery have impressed upon us the fact that all too often a debutant cook will start in enthusiastically on a new dish without ever reading the recipe first. Suddenly an ingredient, or a process, or a time sequence will turn up, and there is astonishment, frustration, and even disaster. We therefore urge you, however much you have cooked, always to read the recipe first, even if the dish is familiar to you. . . . We have not given estimates for the time of preparation, as some people take half an hour to slice three pounds of mushrooms, while others take five minutes.**

1. Read the laboratory exercises *before* coming to class.
2. *Plan* your work so that all experiments will be completed during the assigned laboratory period. A good laboratory student, like a good cook, is one who can do more than one procedure at a time—that is, one who is efficient.
3. Use only the *required* amounts of materials, so that everyone can do the experiment.
4. *Label* all of your experiments with your name, date, and lab section.
5. Even though you will do most exercises with another student, you must become familiar with all parts of each exercise.
6. Keep *accurate* notes and records of your procedures and results so that you can refer to them for future work and tests. Many experiments are set up during one laboratory period and observed for results in the next laboratory period. Your notes are essential to ensure that you perform all the necessary steps and observations.
7. *Demonstrations* will be included in some of the exercises. Study the demonstrations and learn the content.
8. Let your instructor know if you are color-blind; many techniques require discrimination of colors.
9. Keep your cultures current; discard old experiments.
10. *Clean up* your work area when you are finished. Leave the laboratory clean and organized for the next student. Remember:

    Stain and reagent bottles should be returned to their original locations.

    Slides should be washed and put back into the box clean.

    All markings on glassware (e.g., Petri plates and test tubes) should be removed before putting glassware into the marked autoclave trays.

    Glass Petri plates should be placed agar-side down in marked autoclave containers.

    Swabs and pipettes should be placed in the appropriate disinfectant jars or biohazard containers.

    Disposable plasticware should be placed in marked autoclave containers.

    Used paper towels should be discarded.

Biosafety

The most important element for managing microorganisms is strict adherence to standard microbiological practices and techniques, which you will learn during this course. There are four biosafety levels (BSLs) for working with live microorganisms; each BSL consists of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. See Table 1 on page xiii. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the microorganisms, and the laboratory function or activity.

Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no specific facilities other than a sink for hand washing. When standard laboratory practices are not sufficient to control the hazard associated with a particular microorganism, additional measures may be used.

Biosafety Level 2 includes hand washing, and an autoclave must be available. Precautions must be taken for handling and disposing of contaminated needles or sharp instruments. BSL 2 is appropriate when working with human body fluids. A lab coat should be worn.

Biosafety Level 3 is used in laboratories where work is done with pathogens that can be transmitted by the respiratory route. BSL 3 requires special facilities with self-closing, double doors and sealed windows.

Biosafety Level 4 is applicable for work with pathogens that may be transmitted via aerosols and for which there is no vaccine or safety. The BSL 4 facility is generally a separate building with specialized ventilation and waste management systems to prevent release of live pathogens to the environment.

Which biosafety level is your lab? _____

Specific Hazards in the Laboratory

Procedures marked with this safety icon should be performed carefully to minimize risk of exposure to chemicals or fire.

Alcohol

Keep containers of alcohol away from open flames.

Glassware Not Contaminated with Microbial Cultures

1. If you break a glass object, sweep up the pieces with a broom and dustpan. Do not pick up pieces of broken glass with your bare hands.
2. Place broken glass in one of the containers marked for this purpose. The one exception to this rule concerns broken mercury thermometers; consult your instructor if you break a mercury thermometer.

*J. Child, L. Bertholle, and S. Beck. *Mastering the Art of French Cooking*, Vol. 1. New York: Knopf, 1961.

Table 1
Biosafety Levels

Biosafety Level	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Standard microbiological practices	None required	Open benchtop sink
2	BSL1 plus • Limited access • Biohazard warning signs • "Sharps" precautions • Safety manual of waste-decontamination policies	Lab coat; gloves, as needed	BSL1 plus autoclave
3	BSL 2 plus • Controlled access • Decontamination of clothing before laundering	BSL 2 plus protective lab clothing; enter and leave lab through clothing changing and shower rooms	BSL 2 plus self-closing, double-door access
4	BSL 3 plus • Separate building	BSL 3 plus full-body air-supplied, positive pressure personnel suit	BSL 3 plus separate building and decontamination facility

Electrical Equipment

1. The basic rule to follow is this: Electricity and water don't mix. Do not allow water or any water-based solution to come into contact with electrical cords or electrical conductors. Make sure your hands are dry when you handle electrical connectors.
2. If your electrical equipment crackles, snaps, or begins to give off smoke, do not attempt to disconnect it. Call your instructor immediately.

Fire

1. If *gas burns* from a leak in the burner or tubing, turn off the gas.
2. If you have a *smoldering sleeve*, run water on the fabric.
3. If you have a *very small fire*, the best way to put it out is to smother it with a towel or book (not your hand). Smother the fire quickly.
4. If a *larger fire* occurs, such as in a wastebasket or sink, use one of the fire extinguishers in the lab to put it out. Your instructor will demonstrate the use of the fire extinguishers.
5. In case of a *large fire* involving the lab itself, evacuate the room and building according to the following procedure:
   a. Turn off all gas burners, and unplug electrical equipment.
   b. Leave the room and proceed to _____ _____.
   c. It is imperative that you assemble in front of the building so that your instructor can take roll to determine whether anyone is still inside. Do not wander off.

Accidents and First Aid

1. Report all accidents immediately. Your instructor will administer first aid as required.
2. For spills in or near the eyes, use the eyewash immediately.
3. For large spills on your body, use the safety shower.
4. For heat burns, chill the affected part with ice as soon as possible. Call your instructor.
5. Place a bandage on any cut or abrasion.

Power Outage

If the electricity goes off, be sure to turn off your gas jet. When the power is restored, the gas will come back on.

Earthquake

Turn off your gas jet and get under your lab desk during an earthquake. Your instructor will give any necessary evacuation instructions.

Orientation Walkabout

Locate the following items in the lab:

Broom and dustpan	Instructor's desk
Eyewash	Reference books
Fire blanket	Safety shower
Fire extinguisher	To Be Autoclaved area
First-aid cabinet	Biohazard containers
Fume hood	

Special Practices

1. Keep laboratory doors closed when experiments are in progress.
2. The instructor controls access to the laboratory and allows access only to people whose presence is required for program or support purposes.
3. Place contaminated materials that are to be decontaminated at a site away from the laboratory into a durable, leakproof container that is closed before being removed from the laboratory.
4. An insect and rodent control program is in effect.
5. A needle should not be bent, replaced in the sheath, or removed from the syringe following use. Place the needle and syringe promptly in a puncture-resistant container and decontaminate, preferably by autoclaving, before discarding them.
6. Inform your instructor if you are pregnant, are taking immunosuppressive drugs, or have any other medical condition (e.g., diabetes, immune deficiency) that might necessitate special precautions in the laboratory.
7. Potential pathogens used in the exercises in this manual are classified in Class 1 by the U.S. Public Health Service. These bacteria present a minimal hazard and require ordinary aseptic handling conditions (Biosafety Level 1). No special competence or containment is required. These organisms are the following:

   Enterobacter species
   Mycobacterium species
   Proteus species
   Pseudomonas aeruginosa
   Salmonella enterica Typhimurium
   Serratia marcescens
   Staphylococcus species
   Streptococcus species

Laboratory Facilities

1. Interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned.
2. Benchtops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Windows in the laboratory are closed and sealed.
4. An autoclave for decontaminating laboratory wastes is available, preferably within the laboratory.

Contact with Blood and Other Body Fluids

The following procedures should be used by all health care workers, including students, whose activities involve contact with patients or with blood or other body fluids. While these procedures were developed by the CDC* to minimize the risk of transmitting HIV in a health care environment, adherence to these guidelines will minimize transmission of *all* infections.

1. Wear gloves for touching blood and body fluids, mucous membranes, or nonintact skin and for handling items or surfaces soiled with blood or body fluids. Change gloves after contact with each patient.
2. Wash hands and other skin surfaces immediately and thoroughly if they are contaminated with blood or other body fluids.
3. Wear masks and protective eyewear or face shields during procedures that are likely to generate droplets of blood or other body fluids.
4. Wear gowns or aprons during procedures that are likely to generate splashes of blood or other body fluids.
5. Wash hands and other skin surfaces immediately after gloves are removed.
6. Mouthpieces, resuscitation bags, or other ventilation devices should be available for use in areas in which the need for resuscitation is predictable. Emergency mouth-to-mouth resuscitation should be minimized.
7. Health care workers who have exudative lesions or weeping dermatitis should refrain from all direct patient care and from handling patient care equipment.
8. Pregnant health care workers are not known to be at greater risk of contracting HIV infection than health care workers who are not pregnant; however, if a health care worker develops HIV infection during pregnancy, the infant is at risk of infection. Because of this risk, pregnant health care workers should be especially familiar with and strictly adhere to precautions to minimize the risk of HIV transmission.
9. In a laboratory exercise where human blood is used, students should wear gloves or work only with their own blood and should dispose of all slides and blood-contaminated materials immediately after use. Any cuts or scrapes on the skin should be covered with a sterile bandage.

*CDC. "Recommendations for Prevention of HIV Transmission in Health-Care Settings." *MMWR* 38(S2), 1989.

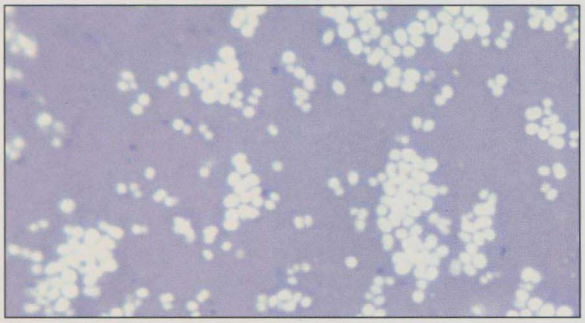
General Safety in the Laboratory

During your microbiology course, you will learn how to safely handle fluids containing microorganisms. Through practice you will be able to perform experiments so that bacteria, fungai, and viruses remain in the desired containers, uncontaminated by microbes in the environment. These techniques, called **aseptic techniques,** will be a vital part of your work if you are going into health care or biotechnology.

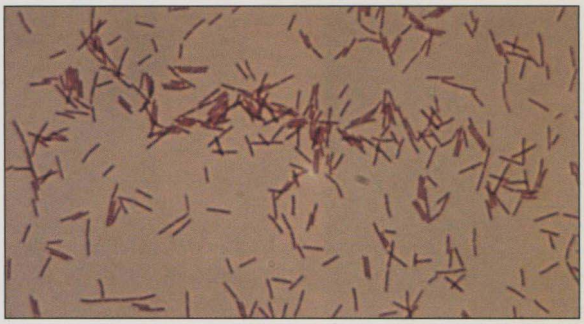
1. Do not eat, drink, smoke, store food, or apply cosmetics in the laboratory.
2. Wear shoes at all times in the laboratory.
3. Tie back long hair.
4. Disinfect work surfaces at the beginning and end of every lab period and after every spill. The disinfectant used in this laboratory is _____.
5. Wash your hands before and after every laboratory period. Because bar soaps may become contaminated, use liquid or powdered soaps.
6. Use mechanical pipetting devices; do not use mouth pipetting.
7. Place a disinfectant-soaked paper towel on the desk while pipetting.
8. Wash your hands immediately and thoroughly if they become contaminated with microorganisms.
9. Cover spilled microbial cultures with paper towels, and saturate the towels with disinfectant. Leave covered for 20 minutes, and then clean up the spill and dispose of the towels.
10. Do not touch broken glassware with your hands; use a broom and dustpan. Place broken glassware contaminated with microbial cultures or body fluids in the To Be Autoclaved container. (See p. xii for what to do with broken glassware that is not contaminated.)
11. Place glassware and slides contaminated with blood, urine, and other body fluids in disinfectant.
12. Work only with your own body fluids and wastes in exercises requiring saliva, urine, blood, or feces, to prevent transmission of disease. The Centers for Disease Control and Prevention (CDC) state that "epidemiologic evidence has implicated only blood, semen, vaginal secretions, and breast milk in transmission of HIV." *Biosafety in Microbiological and Biomedical Laboratories*, www.cdc.gov.
13. Don't perform unauthorized experiments.
14. Don't use equipment without instruction.
15. Don't engage in horseplay in the laboratory.
16. If you got this far in the instructions, you'll probably do well in lab. Enjoy lab and make a new friend.

Procedures marked with this biohazard icon should be performed carefully to minimize the risk of transmitting disease.

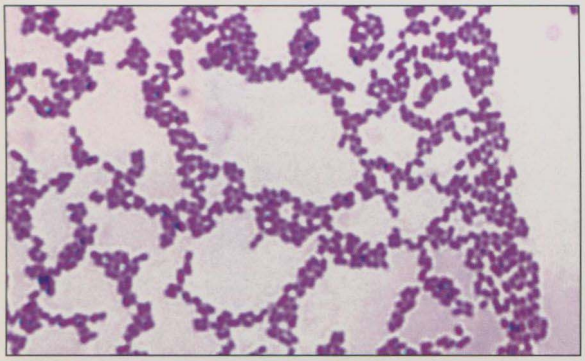
I have read the above laboratory safety rules and agree to abide by them when in the laboratory.
Name: _____ Date: _____



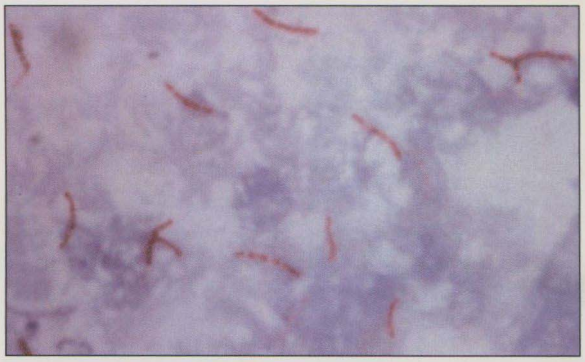
I.1 Negative stain.
Staphylococcus aureus (Exercise 4).



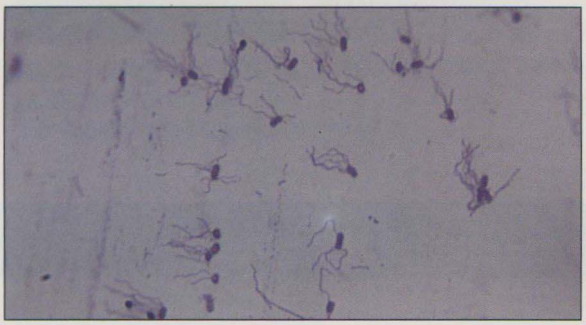
I.2 Gram-negative rods.
Escherichia coli (Exercise 5).



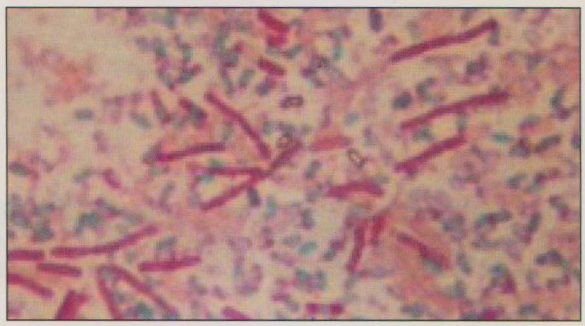
I.3 Gram-positive cocci. *Staphylococcus aureus* (Exercise 5).



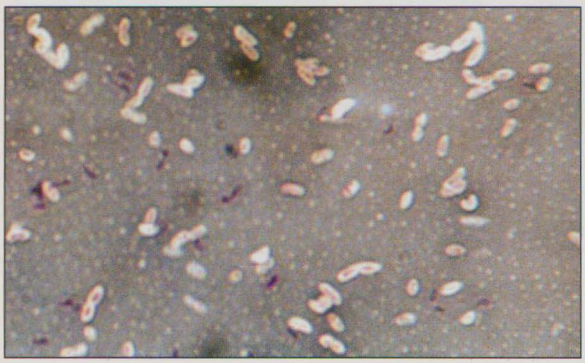
I.4 Acid-fast stain. *Mycobacterium tuberculosis* (Exercise 6).



I.5 Flagella stain. Peritrichous flagella of *Proteus vulgaris* (Exercise 7).

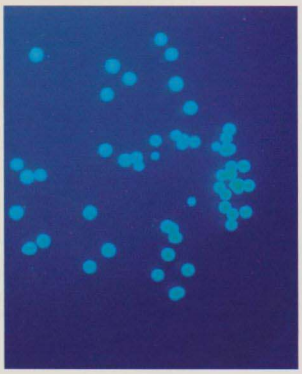


I.6 Endospore stain. *Bacillus* sp. (Exercise 7).

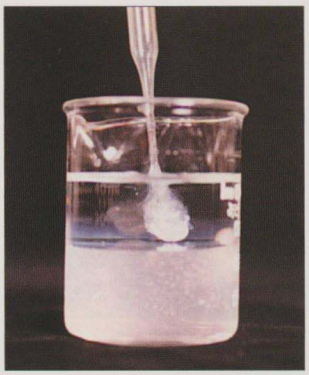


I.7 Capsule stain. *Streptococcus mutans* (Exercise 7).

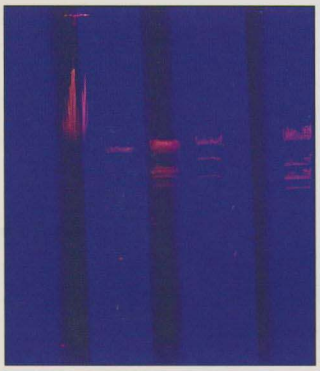
PLATE II BIOTECHNOLOGY



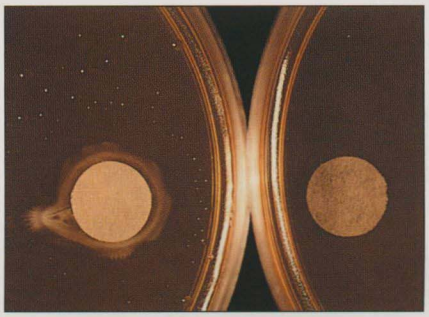
II.1 *E. coli*. The colonies fluoresce under UV light because the cells contain the jellyfish gene for green fluorescent protein (Exercise 27).



II.2 DNA is collected on a glass rod (Exercise 29).

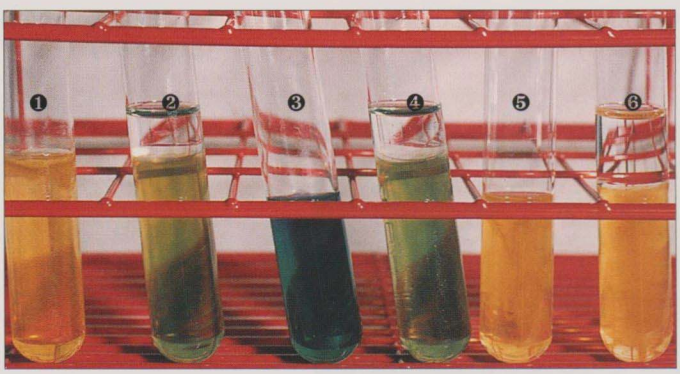


II.3 Restriction enzyme digests of small pieces of DNA. To see the bands, the DNA was stained with ethidium bromide and the gel was illuminated with UV light (Exercise 30).

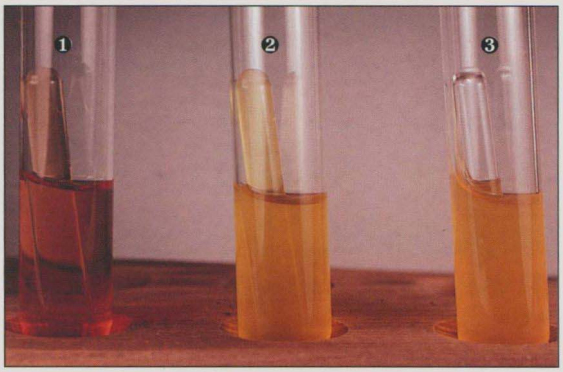


II.4 Ames test. The chemical (ethidium bromide) diffused from the disk on the left and caused the his^- bacteria to revert to his^+ (colonies in the upper left). The his^- cells on the control plate (right) are unable to grow on glucose-minimal salts agar (Exercise 32).

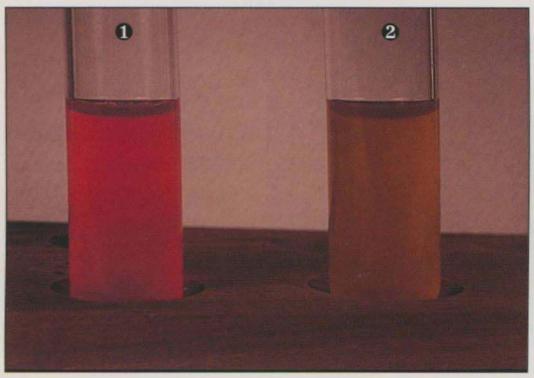
PLATE III MICROBIAL METABOLISM



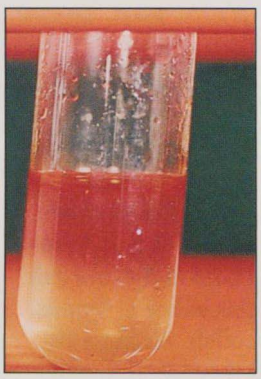
III.1 Reactions in OF-glucose medium. The species in tubes **1** and **2** is an oxidizer. The organism in tubes **3** and **4** does not use glucose. The culture in tubes **5** and **6** is a fermenter (Exercise 13).



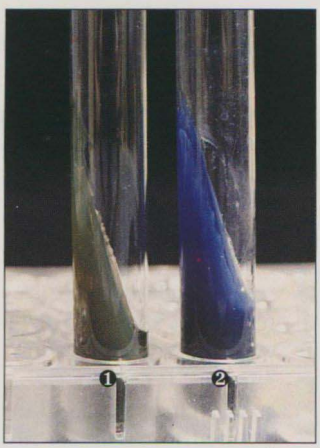
III.2 Reactions in fermentation tubes. **1** is an uninoculated control. Growth and acid production from carbohydrate fermentation are seen in **2.** Acid and gas are produced from fermentation in **3** (Exercise 14).



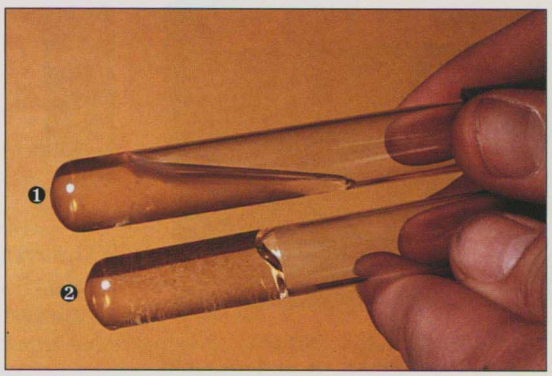
III.3 Methyl red test. Red color (in **1**) after addition of methyl red indicates a positive test. **2** is methyl red–negative (Exercise 14).



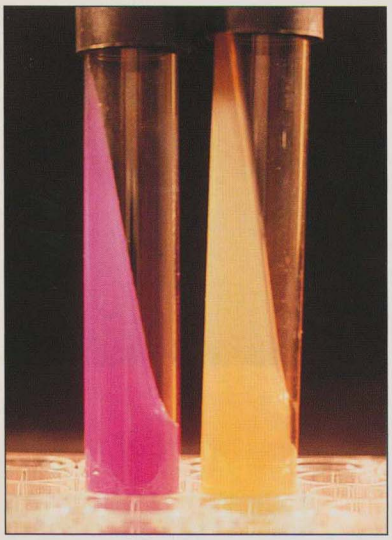
III.4 Voges–Proskauer test. A positive Voges–Proskauer test develops a red color when exposed to oxygen (Exercise 14).



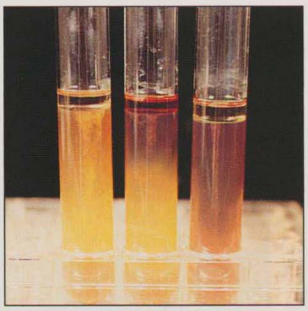
III.5 Citrate test. Utilization of citric acid as the sole carbon source in Simmons citrate agar causes the indicator to turn blue (**2**). **1** is citrate-negative (Exercise 14).



III.6 Gelatin hydrolysis. After hydrolysis (**1**), gelatin remains liquid. **2** is unhydrolyzed gelatin (Exercise 15).

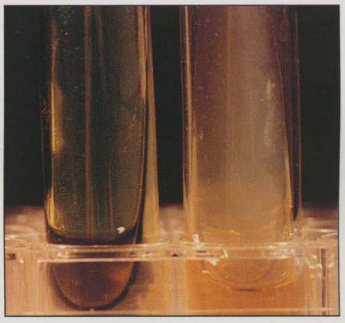


III.7 Urease production. Hydrolysis of urea produces ammonia, which turns the indicator fuchsia (Exercise 15).

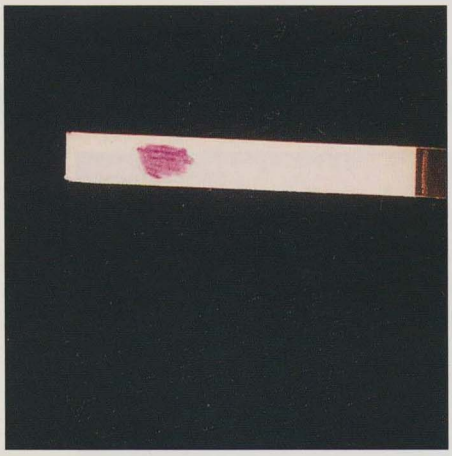


III.8 MIO medium. The culture in the tube on the left is *motile*. The red color of the Kovacs reagent added over the agar indicates *indole* production in the middle tube. Removal of CO_2 from *ornithine* turns the indicator purple, as seen in the tube on the right (Exercise 16).

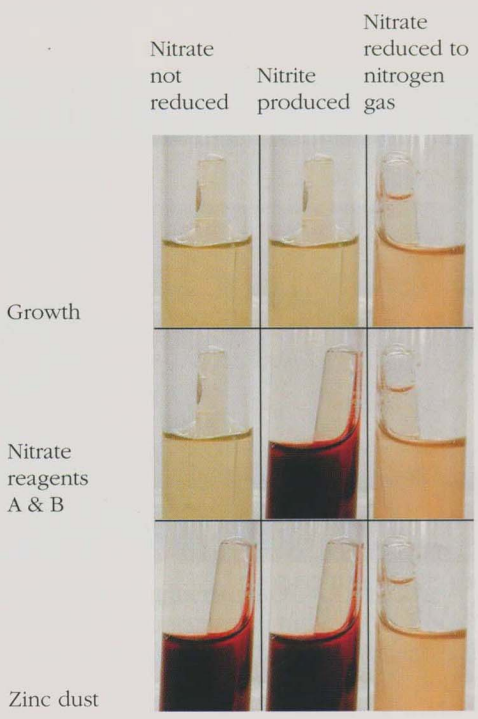
III.9 Peptone iron agar is used to detect the production of H_2S. H_2S produced in the tube will precipitate as ferrous sulfide (Exercise 16).



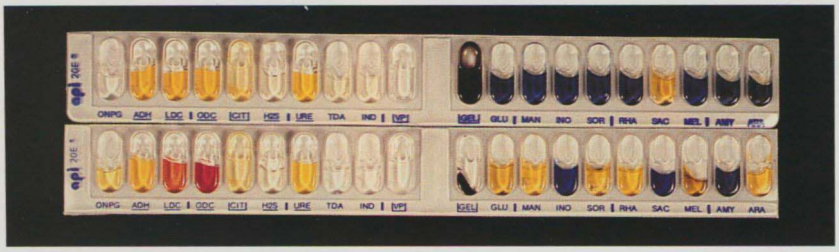
III.10 Phenylalanine deaminase. Removal of the amino group produces an organic acid that forms a green complex with the ferric ion-containing indicator (Exercise 16).



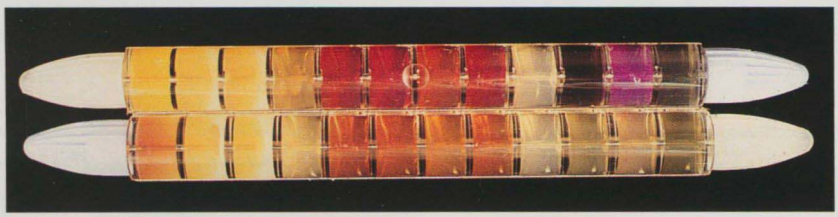
III.11 Oxidase test. Cytochrome oxidase-positive bacteria turn oxidase reagent pink to purple or black (Exercise 17).



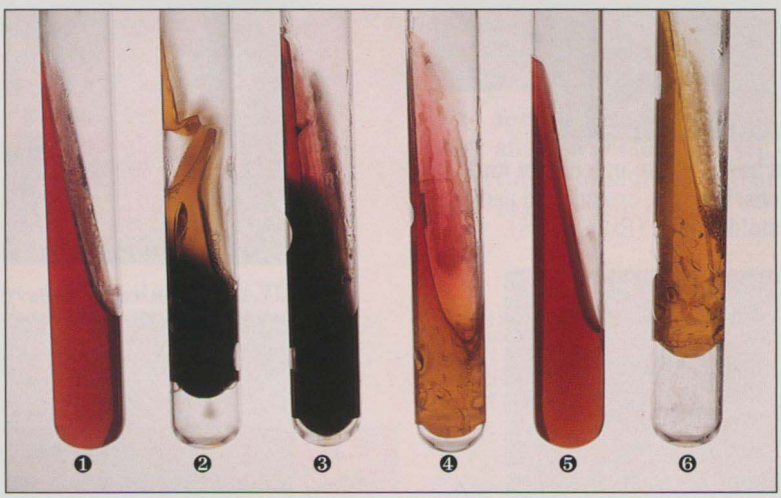
III.12 Nitrate reduction. Nitrate reagent A and nitrate reagent B are added to nitrate broth after incubation to determine nitrate redution. If the broth turns red after addition of nitrate reagents A and B, nitrate ion was reduced to nitrite ion. Zinc dust is added if no color change occurs. If the broth turns red, nitrate ions are present. If nitrates were reduced to nitrogen gas, no color change occurs and the gas should be visible in the Durham (inverted) tube (Exercises 17, 27, and 56).



III.13 Twenty biochemical tests are performed in an API 20E® strip. The top strip is inoculated with *Proteus vulgaris* and the bottom strip with *Escherichia coli* (Exercise 18).



III.14 Fifteen biochemical tests are performed in Enterotube® II. The bottom tube is uninoculated (Exercise 18).



III.15 Reactions in triple sugar iron (TSI) agar. 1 shows growth with no fermentation or hydrogen sulfide (H_2S) production. **2** shows blackening due to H_2S and acid and gas from fermentation of glucose and sucrose and/or lactose. Sucrose and lactose were not fermented in **3**; H_2S production masks the glucose fermentation reaction although gas is produced. Acid and gas are produced from glucose in **4. 5** is uninoculated. **6** shows acid and gas production from glucose and sucrose and/or lactose (Exercise 49).