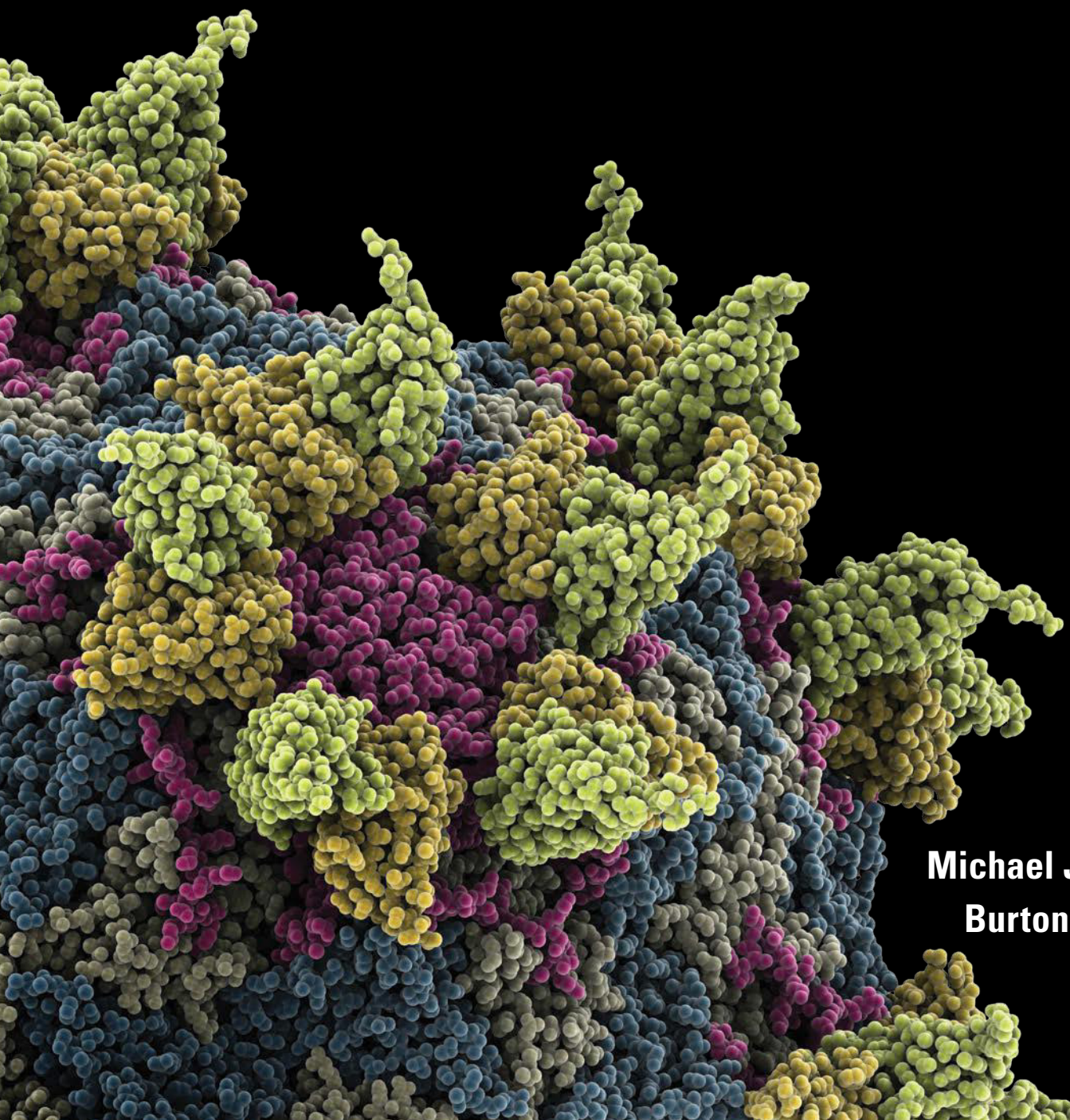
MICROBIOLOGY

Laboratory Theory & Application

BRIEF

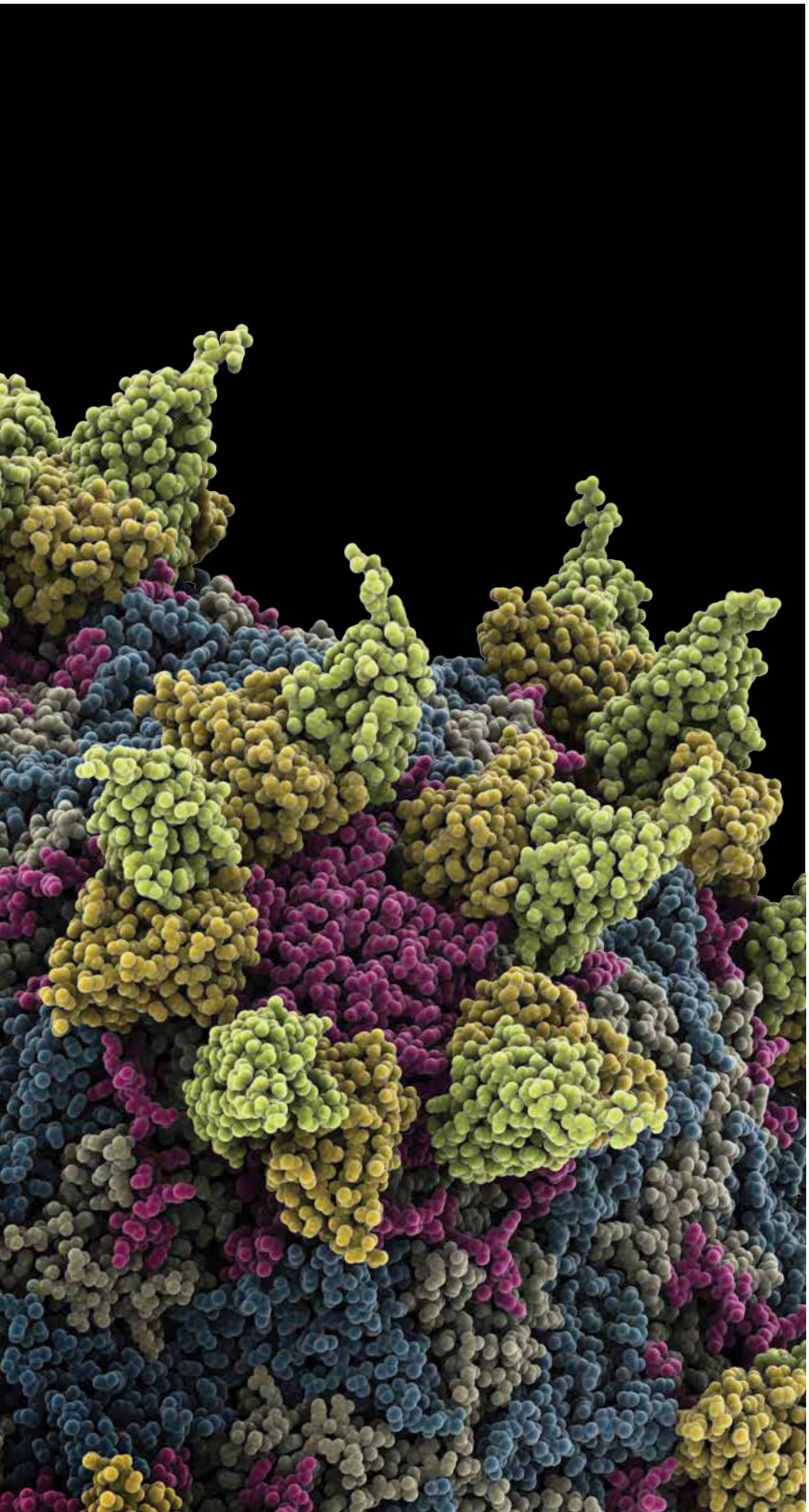
THIRD EDITION



Michael J. Leboffe

Burton E. Pierce

MICROBIOLOGY



Laboratory Theory & Application

BRIEF

THIRD EDITION

Michael J. Leboffe
San Diego City College

Burton E. Pierce

MORTON PUBLISHING

925 W. Kenyon Avenue, Unit 12
Englewood, CO 80110

www.morton-pub.com

Book Team

Publisher	Douglas N. Morton
President	David M. Ferguson
Acquisitions Editor	Marta R. Martins
Project Editor	Rayna S. Bailey
Production Manager	Joanne Saliger
Production Assistant	Will Kelley
Illustrations	Imagineering Media Services, Inc.

Cover image:

Rhinovirus. Molecular model of the antigen-binding fragment (Fab) from a strongly neutralizing antibody bound to a human rhinovirus 14 (HRV-14) particle. This virus consists of a protein capsid enclosing an RNA (ribonucleic acid) genetic code (genome). The rhinovirus infects the upper respiratory tract and is the cause of the common cold. It is spread by coughs and sneezes.

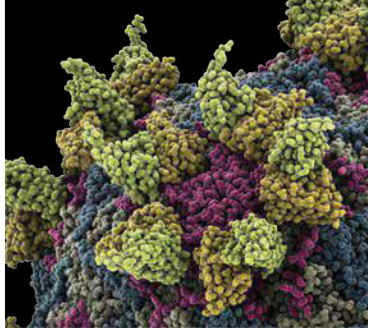
Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

ISBN-10: 1-61731-477-3
ISBN-13: 978-1-61731-477-3

Library of Congress Control Number: 2015919196

PREFACE



Back in college I remember balking at writing a term paper, or even an essay. I never dreamed I'd be writing the preface to a 600+ page, college-level, third edition microbiology laboratory manual. I imagine Dr. Kelly, my General Microbiology professor at San Diego State University, would have felt the same way had she given it any thought. I also figured that hers would be the last microbiology course I'd ever take or need. (I can state with pride, however, I *did* earn a "B.") Silly me, making plans.

Nevertheless, odds and odd career paths be damned: here I am and here it is. America, Land of Opportunity, indeed!

It must be said that "Here *I* am" is only a half-truth. My longtime friend and co-author, Burt Pierce, retired from writing in 2012. As a consequence, the books published since then have become my sole responsibility. But please notice in the descriptions of revisions, deletions, and additions that follow, I use the first person plural pronoun "we" because Burt's influence is still all over this book. Even though the discussions regarding what changes should be made were one-sided in my head, his commitment to quality and not underestimating our audience's abilities continued to influence me. *Microbiology Laboratory Theory & Application*, *Brief,* 3rd edition (hereafter referred to as *MLTAB* 3e), is a spinoff from the larger, more comprehensive *Microbiology Laboratory Theory & Application*, 4th edition (hereafter referred to as *MLTA* 4e).

Whereas *MLTA* 4e targets college-level microbiology courses for science majors, *MLTAB* 3e is designed for use in microbiology courses with more of a medical emphasis and whose students are pursuing an allied health or other health-related career path. Many of the exercises in biotechnology, food and environmental microbiology, and those with a heavy math emphasis have been omitted, but the rigor of those remaining is comparable.

Global Changes

Lab Safety

- From the first edition, we have emphasized laboratory safety and we continue to do so in this edition. Students are reminded to wear a lab coat, eye protection, and gloves in every exercise (when appropriate). They are also reminded to properly dispose of materials (often by telling them to follow the rules of their particular laboratory).
- We continue to emphasize techniques that minimize aerosol production during transfers.
- As in previous editions, BSL-2 organisms are identified in the Materials section of each exercise. Not all strains of a species are BSL-2 and we don't know what strains each college will be using, so we erred on the side of caution in identifying these. We checked ATCC strains and if any were identified as BSL-2 we listed them that way. The instructor may choose to announce that the strain(s) used in their lab are not BSL-2.
- We have continued to reduce the number of BSL-2 organisms used. Where possible, they have been replaced with suitable substitutes that give the same result. In other cases, they have been listed as "optional." In still others, where no replacements are available, they remain. Please pay attention to the organisms you are working with and use appropriate

caution. As always, it is ultimately up to the instructor to use his or her professional judgment and choose organisms that are suitable for the course level and lab facilities of their institution and to each student to follow standard safety guidelines.

- Because some professors may decide to use BSL-2 organisms, we have added a section in the Introduction with recommended BSL-2 procedures.

Pedagogy: Theory, Application, and Instructions

Virtually all lab exercises were rewritten to a greater or lesser degree in an attempt to bring improved clarity to the theory, application, and instructions for each.

Pedagogy: Photos, Micrographs, Artwork, and Tables

One feature of *MLTA* that has pleased adopters of previous editions has been the visual content—photos, micrographs, and art. Upholding our commitment to quality, more than 200 of these elements have been revised or replaced in this edition. Additionally, there are more than 60 brand new photographs, micrographs, and pieces of art.

Pedagogy: Data Sheet Questions

From the first edition of *MLTA* in 2002, the data sheet questions have ranged from simple recall to explanation of procedures, interpretation of data, or extrapolation from what has been covered in the exercise. In this edition, they were examined carefully and in many exercises they have been re-sequenced, reworded, split into parts, or replaced. Some new questions have also been added.

Because there are more exercises in *MLTAB* 3e than can be done in any one-semester course, there is also repetition of certain questions from exercise to exercise. This was done because they address particularly important points and we wanted to ensure that students have to answer them regardless of which exercises are chosen for their course.

Specific and Noteworthy Changes

Following are some highlights of specific topics added or revised in each section.

- **Introduction—Safety and Laboratory Guidelines** A section devoted exclusively to handling BSL-2 organisms has been added, as was a sample "safety contract" to be signed by the student agreeing to comply with safety regulations outlined in this book and as amended by their institution. The topic of controls was also revised.
- **Section 1—Fundamental Skills for the Microbiology Laboratory** In Exercise 1-5 (Streak Plate Methods of Isolation), the T-streak inoculation has been added as an option to the quadrant streak. As a safety precaution, Exercise 1-6 (Spread Plate Method of Isolation) now advises the use of a screw-cap jar to hold the alcohol for flaming the glass rod to minimize the chances of it catching fire.
- **Section 2—Microbial Growth** While the basic exercise protocols are unchanged, each has been rewritten for greater clarity and integration. The biggest change has been in Exercise 2-2 (Colony Morphology). The artwork illustrating colony features was revised and 15 new photos were added, bringing the total up to 44. Some of the new photos were shot through a stereo microscope and illustrate detail (and beauty!) not visible to the naked eye. Lastly, photos are now arranged by the colony features illustrated, such as margin, overall shape, color, etc., rather than randomly as in previous editions.

- **Section 3—Microscopy and Staining** The instructions on general microscope use in Exercise 3-1 (Introduction to the Light Microscope) were enhanced. Exercise 3-3 (Microscopic Examination of Eukaryotic Microbes) was totally reorganized to reflect a more current taxonomy of eukaryotes, and some new photos replaced older ones. Minor revisions were made to the bacterial stain exercises for clarity and safety. New artwork has been added to Exercise 3-6 (Gram Stain) illustrating the mechanism underlying the decolorization step.
- **Section 4—Selective Media** All photos in this section were replaced with better ones. A confusing point for some students in previous editions had been that the photos don't illustrate spot inoculations—and they still don't. The reason we made this choice is that spot inoculations don't illustrate the colors or growth patterns as clearly in photographs as short streaks do.
- **Section 5—Differential Tests** This section was the recipient of many replacement photos and new artwork. It also was treated to sequence changes, with related tests brought together. Additionally, test interpretation tables now address the possibility that a negative result is a false negative.
- **Section 6—Quantitative Techniques** Exercise 6-2 (Standard Plate Count) discusses other methods of counting colonies and explains the rationale for using "Original Sample Volume" rather than "Plate Dilution Factor" in calculations.
- **Section 7—Medical Microbiology** The Theory section of Exercise 7-1 (Snyder Test) was rewritten, and a Gram stain of a tooth scraping replaces a Gram stain of the gumline. Two optional antibiotics have been added to the materials list for the Kirby-Bauer test (Exercise 7-2), and the Theory was rewritten with more detail about the test and its history. There are also optional instructions for using a spectrophotometer in place of a MacFarland standard. Exercise 7-3 (Morbidity and Mortality Weekly Report [MMWR] Assignment) was updated to reflect changes in the CDC website and changes to the list of notifiable diseases. Instructions for Exercise 7-4 (Epidemic Simulation) were rewritten to emphasize the safe execution of the lab. Exercise 7-6 (Multiple Tube Fermentation Method for Total Coliform Determination) was rewritten.
- **Section 8—Microbial Genetics and Serology** Photos of the jellyfish from which the green fluorescent protein gene was obtained are included in Exercise 8-2 (Bacterial Transformation: The pGLO System). An extensive section introducing antigens and antibodies was written and precedes the serology exercises (Exercises 8-4 through 8-6). In addition, Exercise 8-5 (Blood Typing) now explains more thoroughly the genetic and molecular basis for blood types in the ABO system.
- **Section 9—Identification of Unknowns** Most BSL-2 organisms have been removed from the flowcharts in Exercises 9-1 through 9-3. In addition, results were verified and in some cases the flowcharts were modified. Exercise 9-4 (api® 20 E Identification System for *Enterobacteriaceae* and other Gram-Negative Rods) was updated based on the most recent instructions from bioMérieux, Inc., and screen shots of the analytical profile index from api*web* replace the images of its printed counterpart. In Exercise 9-5 (EnteroPluri-*Test*), the Enterotube has been retired and replaced with the EnteroPluri-*Test* system, which is comparable but slightly different than its predecessor. The Streptex Rapid Agglutination test has been removed because of cost and feedback from adopters (but still can be custom published, if desired).

- **Appendices** Artwork in Appendix A (Biochemical Pathways) has been modified greatly. Intersecting pathways have been removed from Figures A.2 (Glycolysis) and A.5 (Entry Step and Citric Acid Cycle), but a new diagram (Fig. A.1, Integrated Metabolism) shows integrated metabolism and references relevant lab exercises for the various pathways. It is designed to show how the biochemical tests (mostly in Section 5) fit into the bigger picture of metabolism. Figure A.6 (Sampling of Fermentation Pathways) has been redrawn and color-coded to indicate specific fermentations. Appendices B, C, and D (transfer methods) have been rewritten to include BSL-2 precautions, and the majority of photos in Appendices B and D have been replaced.

As you proceed through your microbiology lab, please step back and take a moment to marvel at how amazing the microbes you are studying really are and to cultivate an appreciation of them. Remember: you are outnumbered!

All the best,

Mike
La Mesa, CA
December 2015

Acknowledgments

A favorite saying of mine that I picked up from some unknown or long-forgotten source is this: "Many hands make light work." I would like to consider it to be a universal truth, except that another saying, "Too many cooks spoil the broth" also has merit. So, granting that the world is not black and white, but full of nuance and shades of gray, it is still a favorite saying of mine and I try to apply it whenever I can.

When Burt (now retired) and I started writing for Morton Publishing in 1995, we did all the writing, photography, artwork, permissions, most of the proofreading, and a bunch of other tasks required to produce quality, college-level publications. In the last 20 years, Morton Publishing has grown, not only in the number and variety of books they publish, but in their number of employees. The addition of these many hands has made light(er) work for us, and we are very grateful for all they do and have done.

I must start with Doug Morton, who founded Morton Publishing in 1977 with the vision of producing high-quality textbooks at a reasonable price, a business model that has been wildly successful for his company and is still its guiding philosophy. Burt and I are grateful to Doug for seeing potential in our work and giving us a chance and a vehicle with which to present it to the greater college community. Thanks, Doug. You changed our lives.

I am extremely grateful for the support, encouragement, patience, and friendship of President David Ferguson, Vice President of Operations Chrissy DeMier, and Vice President of Sales and Marketing Carter Fenton. These people have been charged with the task of navigating Morton Publishing through the challenging publishing landscape of the early 21st century, and I feel very secure with them at the helm.

Thanks to Marta Martins, Senior Acquisitions Editor, who administered peer reviews of the previous edition and collated the responses into a manageable form that I could respond to easily. She also offered helpful advice/opinions at times when I was wrestling with alternative solutions to problems…er, "challenges." Honestly, I still have problems, but admitting that just dates me, so I'll be trendy and go with "challenges."

Special thanks go to Rayna Bailey, Project Editor. Rayna edited the manuscript, obtained permissions, coordinated the art program, and communicated with virtually everyone associated with this book so I could concentrate on writing. She has also meticulously gone over page proofs several times (which, to me, is akin to having one's fingernails pulled out with pliers) to ensure that the finished product is the absolute best it can be. Her positive attitude and gentle prodding kept me on task and moving forward. This is the fourth book Rayna and I have worked on since she started at Morton five years ago. If she had been working on our books from the beginning when Burt and I were performing the tasks she now performs, her contributions would have merited co-authorship!

The production team at Morton has once again done a masterful job in designing this book with their customary artistic flair. Art meets science at Morton Publishing! Thanks to Joanne Saliger, Production Manager, and Will Kelley, Production Assistant, for applying their talents to our book. Will not only did the layout and design, he also modified artwork and sometimes created it from scratch, a difficult task considering he was working with technical images represented by my freehand artistic style of scribbles, cross-outs, and arrows! Well done, Will!

Thanks to Scott Day, Sales Manager, and all the sales representatives who meet potential adopters and present my books in the best light possible. Without them, my garage would be full of unsold books! Their job is a difficult one and I appreciate their efforts more than they can know.

As I get older, I have found I'm more productive if I remove myself from my daily distractions, so I spent quite a bit of time working on this edition in the Morton offices. I extend my heartfelt gratitude to everyone who works there for embracing me as one of their own during my temporary occupancy of six different office spaces over the last year. Visit **www.morton-pub.com** and meet all of these people!

Other non-Morton contributors that rounded out the book team are Imagineeringart Inc., of Toronto, ON, who rendered much of the beautiful artwork; Carolyn Acheson, who compiled the index; and Trina Lambert, who applied a fresh pair of proofreader's eyes to the final product. Thanks to all of you.

Thanks are also due to my colleagues at San Diego City College—past and present. Current full-time faculty Jake Brashears, Jennifer Chambers, Roya Lahijani, Erin Rempala, Dave Singer, and Gary Wisehart tolerated my pinball-like presence in the hallways as I raced from classes, office hours, and meetings between writing obligations. "Hello!" became an in-depth conversation over the last year. So now, let's talk sometime. Soon.

City College microbiology instructors Tom Kaido, Sabine Kurz-Camcho, Martha Myers, and Brett Pickett used previous editions of this book in their classes, offered suggestions, and provided me with a sounding board for new ideas, as did my San Diego State University teaching interns Heather Heinz and Polly Parks. Dean (also friend and former office partner) of the School of Engineering & Technologies, Mathematics, Sciences, and Nursing, Minou Spradley, oversaw the process of my renting San Diego City College lab facilities. (These things go so smoothly when I let someone else handle them!)

Last but not least, biology lab technicians Ryan McWey, Deb Reed, Laura Steininger, and Muu Vu were always ready to cheerfully assist me in finding materials I needed that I should have learned the location of years ago. Thank you, one and all.

I engaged in many conversations about safety in the microbiology teaching laboratory with colleagues Marlene DeMers and Tom Gibson, both formerly of San Diego State University. They had a profound influence on my presentation of that topic in this edition. Thanks, you two. Enjoy your retirements!

I also want to recognize the contributions of hand models Kadija Amba, Diana Carrillo, Heather Heinz, Anita Hettena, Alicia Leboffe, Nathan Leboffe, Deb Reed, Burt Pierce, Carla Sweet, Rick Tenorio, and Gary Wisehart. I know which hands are yours and I get a warm, fuzzy feeling remembering each photograph and knowing which hands belong to whom even if the majority of readers probably don't notice the differences. Oh, and just to

show that I'm keeping up with the times, there are also a couple of "selfies" in the book. See if you can find them…

Thanks to the reviewers of *Microbiology Laboratory Theory and Application,* 3e, who provided me with comprehensive feedback and many useful suggestions for revising that book into a fourth edition, from which much of this book is derived. They are: Patricia Clinard Alfing, Davidson County Community College; Richard Adler, University of Michigan–Dearborn; Amy Warenda Czura, Suffolk County Community College–Eastern Campus; Timothy Ladd, Millersville University; and Jeanette M. Loutsch, University of Science and Arts of Oklahoma.

Probably most of all, Burt Pierce, my retired former co-author, deserves recognition because without his contributions there wouldn't have been a first edition of any of our books, much less third and fourth editions. His contributions and influence are still vital parts of this book. Our creative strengths overlapped enough that we were able to see a common vision for our books, but more importantly our individual strengths complemented the other's, making our "whole" greater than the sum of our parts.

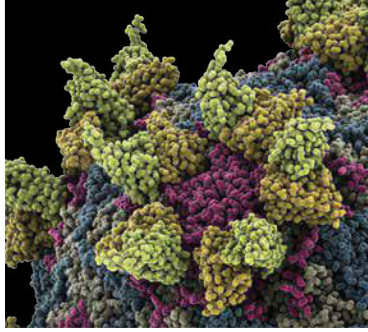
To all the others: There have been so many who have contributed in various ways over four editions of *Microbiology Laboratory Theory and Application (MLTA)*, two editions of *MLTA Brief*, four editions of the *Photographic Atlas for the Microbiology Laboratory*, and four editions of *Exercises for the Microbiology Laboratory* that it has become impractical to identify everyone individually, but your collective efforts have not been forgotten and continue to be appreciated. One big, hearty "Thanks" to you all.

Many hands…indeed.

Dedication

There's no "I" in team, and the Leboffe and Pierce microbiology atlases and lab manuals published by Morton since 1995 have always been the product of teamwork, beginning with two members and expanding upward to its current level of a dozen or so. Although he is retired and no longer an active team member (instead working on his tan in Oregon), I dedicate this book to my longtime friend, San Diego City College colleague, and co-author Burton Pierce. Burt, your work ethic, attention to detail, dedication to doing things "the right way," and sense of humor continue to influence me. There may be no "I" in "team," but there is a "T" in Burt. Thank you for all you've done.

CONTENTS



INTRODUCTION

Safety and Laboratory Guidelines

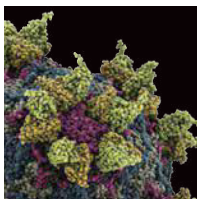
We hope that you find microbiology lab to be an interesting and exciting experience, but at the outset you must be made aware of some potential hazards. Improper handling of chemicals, equipment, and/or microbial cultures is dangerous and can result in injury or infection. Safety with lab equipment will be addressed when you first use that specific piece of equipment, as will specific examples of chemical safety. Our main concern here is to introduce you to safe handling and disposal of microbes.[1]

Because microorganisms present varying degrees of risk to laboratory personnel (oneself, other students, technicians, and faculty), people outside the laboratory, and the environment, microbial cultures must be handled safely. Classifying microbes into four biosafety levels (BSLs) provides a set of minimum standards for laboratory practices, facilities, and equipment to be used when handling organisms at each level. These biosafety levels, defined in the U.S. government publication, *Biosafety in Microbiological and Biomedical Laboratories,* 5th edition (2009), are summarized below and in Table I-1. For complete information, readers are referred to the original document.

BSL-1: Organisms do not typically cause disease in healthy individuals and present a minimal threat to the environment and lab personnel. Standard microbiological practices are adequate. These microbes may be handled in the open, and no special containment equipment is required. Examples include *Bacillus subtilis*, *Escherichia coli* (most strains), *Rhodospirillum rubrum*, and *Lactobacillus acidophilus*.

BSL-2: Organisms are commonly encountered in the community and present a moderate environmental and/or health hazard. These organisms are associated with a variety of human diseases, most of which can be successfully treated if identified in a timely manner. The infection routes of primary concern are ingestion, inhalation, or penetration of the skin (percutaneous). Individuals performing work prone to splashes or aerosol generation (even though these organisms are not generally known to be transmitted by aerosols) should work in a biological safety cabinet (BSC, Fig. I.1). Otherwise, laboratory work may be done using standard microbiological practices. Examples include *Salmonella*, *Staphylococcus aureus*, *Clostridium dificile*, and *Borrelia burgdorferi*.

[1] Your instructor may augment or revise these guidelines to fit the conventions of your laboratory.



Biosafety Cabinets

Biosafety cabinets are classified into three categories, all of which draw air into the cabinet to minimize microbial contamination back into the room.

Class I BSCs resemble chemical fume hoods with a protective glass in the front, but have a HEPA filter along the exhaust path to prevent microbes from entering the environment.

Class II BSCs use laminar airflow, which minimizes turbulence, and HEPA filters to protect the user and materials within the cabinet from contamination. The exhaust passes through a HEPA filter before its release into the environment.

Class III BSCs are completely sealed and gas tight with a fixed viewing window. Incoming air passes through a HEPA filter, whereas materials to be handled are passed through a double-door system where the intermediate compartment is an autoclave. (Other methods may also be used, but accomplish the same purpose.) Exhaust air passes through two HEPA filters or a HEPA filter and an air incinerator. Materials are handled with arm-length, heavy-duty gloves built into the wall of the cabinet. ■

I.1 Biological Safety Cabinet (BSC) in a Teaching Laboratory ■
In this Class II BSC, air is drawn in from the room and is passed through a HEPA filter prior to release into the environment. This airflow pattern is designed to keep aerosolized microbes from escaping from the cabinet. The microbiologist is pipetting a culture. When the BSC is not in use at the end of the day, an ultraviolet light is turned on to sterilize the air and the work surface.
(San Diego County Public Health Laboratory)

BSL-3: Organisms are of local or exotic origin and are associated with respiratory transmission and serious or lethal diseases where treatment and/or vaccines may or may not be available. Special ventilation systems are used to prevent aerosol transmission out of the laboratory, and access to the lab is restricted. Specially trained personnel handle microbes in a Class II or III BSC, not on the open bench. Examples include *Bacillus anthracis*, *Mycobacterium tuberculosis*, and West Nile virus.

BSL-4: Organisms have a great potential for lethal infection. Inhalation of infectious aerosols, exposure to infectious droplets, and autoinoculation are of primary concern. The lab is isolated from other facilities, and access is strictly controlled. Ventilation and waste management are under rigid control to prevent release of the microbial agents to the environment. Specially trained personnel perform transfers in Class III BSCs. Class II BSCs may be used as long as personnel wear positive pressure, one-piece body suits with a life-support system. Examples include agents causing hemorrhagic diseases, such as Ebola, Marburg, and Lassa fever viruses.

The microorganisms used in introductory microbiology courses depend on the institution, objectives of the course, and student preparation. Most introductory courses use organisms that may be handled at BSL-1 and BSL-2 levels so we have followed that practice in designing this set of exercises.

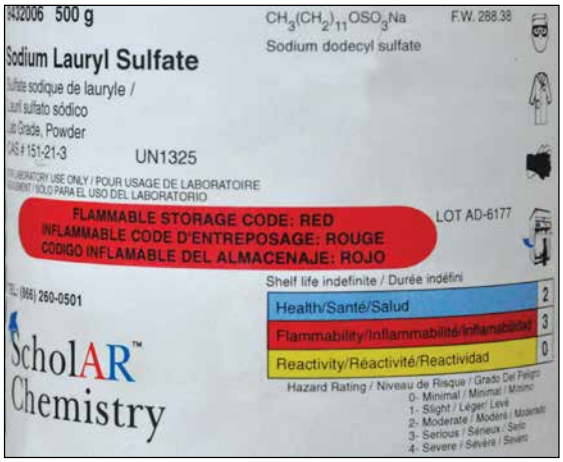
Following are general safety rules to reduce the chance of injury or infection to you and to others, both inside and outside the laboratory. Although both BSL-1 and BSL-2 guidelines are listed, we believe it is best to err on the side of caution and that students should learn and practice the safest level of standards (relative to the organisms they are likely to encounter) at all times. Please follow these and any other safety guidelines required by your college.

Chemical safety is also important in a microbiology laboratory. Be aware of the hazards presented by the chemicals you are handling. Containers should be labeled with a standard set of precautions as seen in Figure I.2. Numbers are assigned to the degree of health, fire, and reactivity hazard posed by the chemical. On stickers placed by the laboratory, there also is a space to enter specific hazards, such as acid, corrosive, and radioactivity.

TABLE I-1 **Summary of Recommended Biosafety Levels for Infectious Agents**

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	■ Not known to consistently cause disease in healthy adults ■ e.g., *Lactobacillus casei*, *Bacillus subtilis*, *Rhizobium leguminosarum*	■ Standard microbiological practices	■ Primary barriers: none required ■ Personal Protective Equipment (PPE): laboratory coats and gloves; eye, face protection as needed	■ Laboratory bench practices and sink required
2	■ Agents associated with human disease; treatments and/or vaccines are usually available ■ Routes of transmission include percutaneous injury, ingestion, and mucous membrane exposure ■ e.g., *Staphylococcus aureus*, *Salmonella enterica*, measles virus	**BSL-1 practices plus:** ■ Limited lab access ■ Biohazard warning signs ■ "Sharps" precautions ■ Biosafety manual defining any needed waste decontamination	■ Primary barriers: Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials ■ PPEs: laboratory coats, gloves, face protection as needed	**BSL-1 plus:** ■ Autoclave available
3	■ Indigenous or exotic agents with potential for aerosol transmission ■ Disease may have serious or lethal consequences; treatments and/or vaccines may be available ■ e.g., *Yersinia pestis*, *Mycobacterium tuberculosis*, rabies virus	**BSL-2 practices plus:** ■ Controlled lab access ■ Decontamination of all waste ■ Decontamination of all lab clothing before laundering ■ Baseline serum	■ Primary barriers: Class I or II BSCs or other physical containment devices used for all manipulations of agents ■ PPEs: protective lab clothing, gloves, respiratory protection as needed	**BSL-2 plus:** ■ Physical separation from access corridors ■ Access to self-closing double door ■ Exhausted air not recirculated ■ Negative airflow into laboratory
4	■ Dangerous/exotic agents that pose high risk of life-threatening disease, aerosol-transmitted lab infections, or related agents with unknown risk of transmission; treatment and vaccines are unavailable ■ e.g., Ebola virus, Lassa virus	**BSL-3 practices plus:** ■ Clothing change before entering ■ Shower on exit ■ All material decontaminated on exit from facility	■ Primary barriers: all procedures conducted in Class III BSCs or Class I or II BSCs *in combination with* full-body, air-supplied, positive pressure personnel suit	**BSL-3 plus:** ■ Separate building or isolated zone ■ Dedicated supply and exhaust vacuum, and decon system ■ Other requirements outlined in the text

Source: Adapted from *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition (Washington, DC: U.S. Government Printing Office, 2007).



I.2 Chemical Hazard Label ■ The blue, red, and yellow boxes on this label indicate the health, flammability, and reactivity levels, respectively, of the compound sodium dodecyl sulfate (SDS). The colors are standardized (regardless of the chemical) and each is assigned a number between "0" (minimal) and "4" (severe) as shown in the legend at the lower right. SDS presents a moderate health hazard, is very flammable, but minimally reactive. Also notice the icons in the upper right, which indicate (from top to bottom) that this compound should be handled with eye protection, a lab coat, gloves, and adequate ventilation. Be aware of these warnings and comply with the associated cautions for all chemicals you handle in lab.

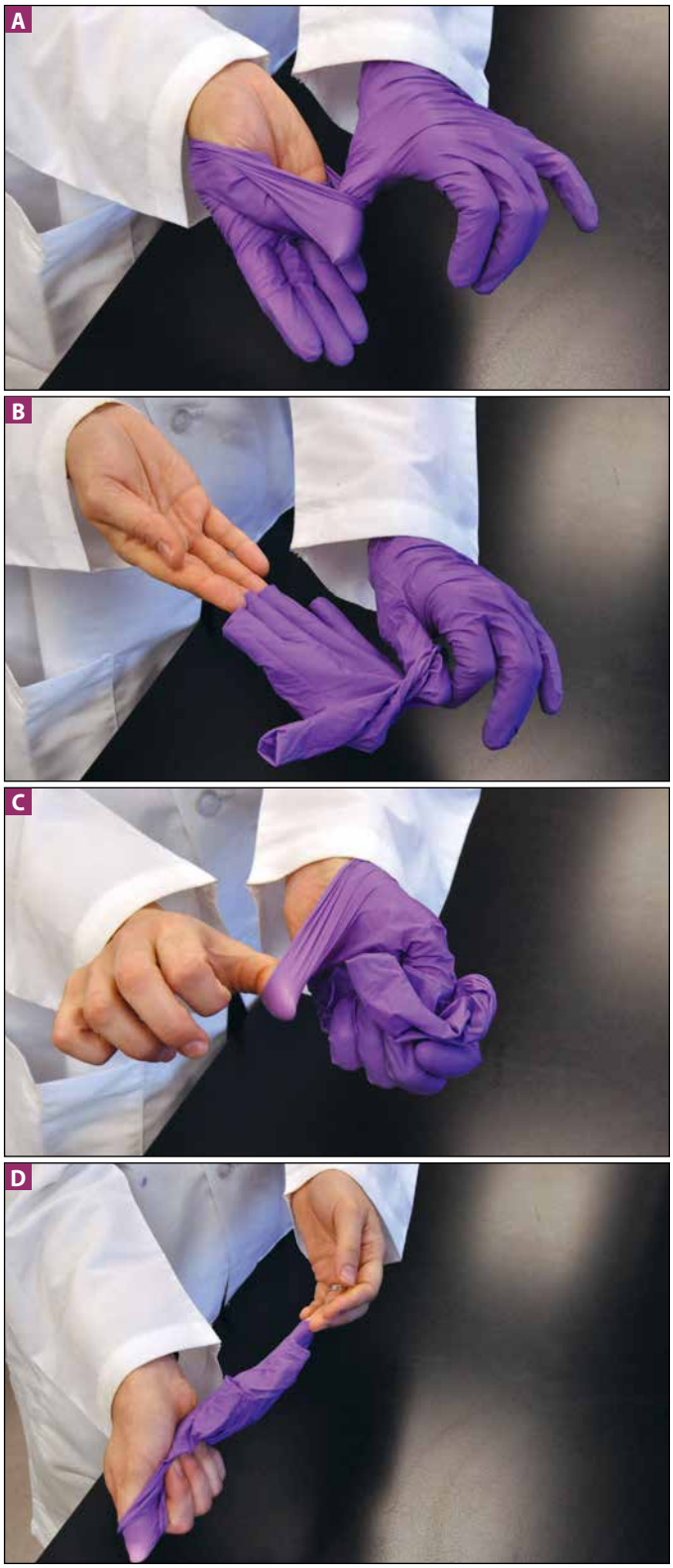
Student Conduct

- To reduce the risk of infection, do not smoke, eat, drink, or bring food or drinks into the laboratory room—even if lab work is not being done at the time.
- Do not apply cosmetics or handle contact lenses in the laboratory.
- Wash your hands *thoroughly* with soap and water after handling living microbes and before leaving the laboratory each day. Also, wash your hands after removing gloves.
- Do not remove any organisms or chemicals from the laboratory.
- Lab time is precious, so come to lab prepared for that day's work. Figuring out what to do as you go is likely to produce confusion and accidents.
- Work carefully and methodically. Do not hurry through any laboratory procedure.

Basic Laboratory Safety

- Wear protective clothing (i.e., a lab coat) in the laboratory when handling microbes. Remove the coat prior to leaving the lab and autoclave it regularly (Fig. I.3). Do not take it home for washing until it has been autoclaved.
- Wear only closed-toe shoes in the laboratory.
- Wear eye protection whenever you are handling microbes or chemicals (especially during heating) even if you wear glasses or contacts (Fig. I.3).
- Turn off your Bunsen burner when it is not in use. In addition to being a fire and safety hazard, it is an unnecessary source of heat in the room.
- Tie back long hair, because it is a potential source of contamination as well as a likely target for fire.
- If you are feeling ill, go home. A microbiology laboratory is not a safe place if you are ill.
- If you are pregnant, immune compromised, or are taking immunosuppressant drugs, please see the instructor. It may be in your best long-term interests to postpone taking this class. Discuss your options with your instructor.
- If it is your lab's practice to wear disposable gloves while handling microorganisms (and this is becoming the norm), be sure to remove them each time you leave the laboratory. The proper method for removal is with the thumb under the cuff of the other hand's glove and turning it inside out without snapping it (Fig. I.4). Gloves should then be disposed of in the container for contaminated materials. Finally, wash your hands.
- Wear disposable gloves while staining microbes and handling blood products—plasma, serum, antiserum, or whole blood. Handling blood can be hazardous, even if you are wearing gloves. Consult your instructor before attempting to work with any blood products.
- Use an antiseptic (e.g., Betadine®) on your skin if it is exposed to a spill containing microorganisms. Your instructor will tell you which antiseptic you will be using. Have a lab partner turn on the water for you if your hands have been contaminated.
- Never pipette by mouth. Always use a mechanical pipettor (Fig. C.1, Appendix C).
- Dispose of broken glass or any other item that could puncture an autoclave bag (contaminated or not) in an appropriate sharps or broken-glass container (Fig. I.5).
- Use a fume hood to perform any work involving highly volatile chemicals or stains that need to be heated.
- Find the first-aid kit, and make a mental note of its location.
- Find the fire blanket, shower, and fire extinguisher, note their locations, and develop a plan for how to access them in an emergency.
- Find the eyewash basin, learn how to operate it, and remember its location.

I.3 Safety First ■ This student is prepared to work safely with microorganisms. The lab area is uncluttered, tubes are upright in a test tube rack, and the flame is accessible but not in the way. The student is wearing a protective lab coat, gloves, and goggles, all of which are to be removed prior to leaving the laboratory. Not all procedures require gloves and eye protection. Your instructor will advise you as to the standards in your laboratory.



I.4 Proper Glove Removal ■ Protective gloves fit tightly around the hands and must be removed in such a way to limit their snapping, which produces aerosols. (**A**) Begin by hooking the thumb of one hand under the cuff of the glove on the other hand. (**B**) Next, roll the glove off the hand so that it turns inside out. (**C**) Take the removed glove in the hand that still has a glove on it and repeat the process. (**D**) As you roll the second glove off your hand, the first glove will end up inside the second glove, which also ends up inside out. Upon completion, all contaminated surfaces are inside the second glove.

Reducing Contamination of Self, Others, Cultures, and the Environment

- Wipe the desktop with a disinfectant (e.g., Coverage® or 10% chlorine bleach) before *and* after each lab period. Never assume that the class before you disinfected the work area. An appropriate disinfectant will be supplied. Allow the disinfectant to evaporate; do not wipe it dry.
- Never lay culture tubes on the table; they always should remain upright in a tube holder (Fig. I.3). Even solid media tubes contain moisture or condensation that may leak out and contaminate everything it contacts.
- Cover any culture spills with paper towels. Soak the towels immediately with disinfectant and allow them to stand for 20 minutes. Report the spill to your instructor. When you are finished, place the towels in the container designated for autoclaving.
  - If the culture spills on you, remain where you are, do not touch anything, and have your lab partner notify the instructor. Your instructor will advise your group on how to handle the spill.
  - If you get a microbial culture in your eyes IMMEDIATELY have a lab partner lead you to the eyewash basin and rinse your eyes for at least 15 minutes. Time is of the essence for eye contamination. Your lab partners must notify the instructor of the spill and your situation, and follow his/her instructions for handling your care and the spill.

I.5 Sharps Container ■ Needles, glass, and other contaminated items that can penetrate the skin or an autoclave bag should be disposed of in a sharps container. Do not fill above the dashed black line. Notice the autoclave tape in the lower left. The white stripes will turn black after proper autoclaving. Above the autoclave tape is the address of the institution that produced the biohazardous waste.

- Keep all nonessential books and papers off the desk. A cluttered lab table is an invitation for an accident that may contaminate your expensive school supplies. Your instructor will advise you where to store these items.
  - Cell phones, tablets, computers, and other electronic devices must never be on the lab table when working with microbes. Contamination of these items can be a health hazard as well as expensive.
- When pipetting microbial cultures, place a disinfectant-soaked towel on the work area. This reduces contamination and possible aerosols if a drop escapes from the pipette and hits the tabletop.

Guidelines Governing Handling of BSL-2 Organisms

Following is a list of precautions for handling BSL-2 organisms. A number of these were covered previously, but are repeated here along with the new ones and now are considered "highly recommended." It is also highly recommended that before students are allowed to handle BSL-2 organisms, they should demonstrate skill in handling BSL-1 organisms safely.

Your college may have other guidelines for handling BSL-2 organisms and other standards of practice may be adopted during the lifetime of this book, so pay attention to any announcements your lab instructor may give. And, as always, it is the instructor's responsibility to choose organisms and lab exercises appropriate to the skill level of his/her students.

- The lab instructor should announce to the class when BSL-2 precautions are in effect. Your college may also institute a mechanism for identifying BSL-2 cultures, such as using red caps on culture tubes. Be aware of any conventions used by your lab.
- Access to the lab must be limited once work with the BSL-2 organisms has begun. Doors and windows must be closed.
- All unnecessary materials (books, backpacks, etc.—in other words, anything you plan on taking home) must be kept off the work surface and safe from aerial contamination and spills. Your lab will have a designated place for their storage.
- Disinfect your lab table top before and after the activity. Allow the disinfectant to air-dry.
- A lab coat, gloves, and eye protection must be worn throughout the handling of BSL-2 organisms. Do not touch any items not directly involved in the lab activity. Properly remove and dispose of the gloves when finished. (See Fig. I.4 for proper glove removal.)
- Writing utensils must be "lab dedicated," that is, they do not leave the lab until they have been disinfected. It is best to wipe them down with disinfectant after use but keep them in your lab drawer for the entire semester. There is no sense in risking contamination of multiple pens and pencils by using different ones each day.
- Electronic devices (e.g., laptops, tablets, cell phones) must be kept off the work surface and stored in an area protected from spills and aerial contamination.
- Use page covers to protect your lab exercise pages while performing the activity. Once your work is completed, disinfect the page covers, wash your hands, and remove the pages. When dry, the page covers can be stored in your locker and reused. The lab book pages can be taken home.
- It is best to use a photocopy of your data sheet to record results. Once you have decontaminated the area, it can be photographed and the results can be transcribed to the original data sheet.[2] Dispose of the photocopy in an appropriate biohazard container. It must not leave the lab.
- Minimizing aerosol production during the transfer of microorganisms is important and the instructions in Section 1 address methods for doing so. However, here is a summary of recommended practices specific to BSL-2 organisms.
  - Wire loops and needles should be incinerated in an enclosed electric incinerator (refer to Section 1, Fig. 1.15, p. 34). Sterilization with an open flame should be avoided because of its potential for aerosol production.
  - An alternative to the electric incinerator is to perform transfers with sterile disposable loops or sterile wooden sticks. Both will work for transferring from solid or liquid media. After each use, they should be put in a disposal container (such as a can) contaminated end down, submerged in about 2 cm of disinfectant. When finished with the exercise, the can should be put in a bin for autoclaving or other disposal receptacle your lab uses. Alternatively, sticks and disposable loops can be put in a sharps container if that is your lab's practice.
  - Pipetting must be done in such a way that the last drop is *not* "blown out" unless a Petri dish lid is covering a plate. Appendices C and D give specific instructions for pipetting BSL-2 organisms.

[2] Alternatively, if your lab has a dedicated document scanner, your completed data sheet can be scanned and emailed to your instructor for grading, with the original being retained in the lab. Thanks to Dr. Brian Gray, York College of Pennsylvania, for sharing this idea with me at the 2015 ASMCUE conference in Austin, TX.

- Clean up after the activity.
  - For incubation, place tubed cultures in a rack. For plate cultures, it is best to tape the lids on each plate individually by taping around the edge of the plate rather than taping around the base and lid (Fig. I.6). This allows viewing of the agar surface without having to remove the tape.
  - Wipe down the work surface, writing utensils, and page covers (if any) with disinfectant and let them air-dry. Only after the disinfectant has dried is it okay to place items to be removed from lab on your table.
  - Remove your gloves, eye protection, and lab coat and store them according to your lab's conventions.
  - Wash your hands thoroughly.
- Photograph or scan your data sheet and store or dispose of it properly.

I.6 Taping Plates ■ It is a good idea to tape the lid of a Petri dish to its base when incubating or storing it, especially if it contains a BSL-2 organism or a culture of unknown microbes. (**A**) One method is to wrap a length of tape around the lid and then use your thumb to press it firmly to the base. (**B**) When finished, the entire agar surface is visible and not obscured by any tape, making lid removal unneccessary to view colonies.

Disposing of Contaminated Materials

In most instances, the preferred method of decontaminating microbiological waste and reusable equipment is the autoclave (Fig. I.7).

- Remove all labels from tube cultures and other contaminated *reusable* items and place them in the designated autoclave container. This will likely be an open autoclave pan to enable cleaning the tubes and other items following sterilization.
- Dispose of plate cultures (if plastic Petri dishes are used) and other contaminated non-sharp *disposable* items in the designated autoclave container, such as an autoclave bag (Fig. I.8). Petri dishes should be taped closed. (***Note:*** Autoclave containers are designed to be autoclaved, permanently closed, and discarded. Therefore, do not place reusable and nonreusable items in the same container.)
- Dispose of all blood product samples and disposable gloves in the container designated for autoclaving.
- Place used microscope slides of bacteria in a sharps container designated for autoclaving, or soak them in disinfectant solution for at least 30 minutes before cleaning or discarding them. Follow your laboratory guidelines for disposing of glass.
- Place contaminated broken glass and other sharp objects (anything likely to puncture an autoclave bag) in a sharps container designated for autoclaving (Fig. I.5). Uncontaminated broken glass does not need to be autoclaved, but should be disposed of in a specialized broken glass container.

I.7 An Autoclave ■ Media, cultures, and equipment to be sterilized are placed in the basket of the autoclave. Steam heat at a temperature of 121°C (produced at atmospheric pressure plus 15 psi) for 15 minutes is effective at killing even bacterial spores. Some items that cannot withstand the heat, or have irregular surfaces that prevent uniform contact with the steam, are sterilized by other means.

I.8 An Autoclave Bag ■ Nonreusable items (such as plastic Petri dishes) are placed in an autoclave bag for decontamination. Petri dishes should be taped closed. Do not overfill or place sharp objects in the bag. Notice the autoclave tape at the middle right. The white stripes will turn black after proper autoclaving. At the lower right is the address of the institution that produced the biohazardous waste.

Student Compliance with Laboratory Safety Regulations

Your institution may augment the safety regulations outlined above or compile a list tailored to your laboratory. In either case, these constitute a Laboratory Safety Statement. It is highly recommended that students verify that they have read and understand these safety regulations, agree to comply with them, and are aware of noncompliance consequences by signing a statement to that effect. The American Society for Microbiology has produced a publication (Emmert et al., 2012) that includes a sample Laboratory Safety Statement and a student signature page. The signature portion in a slightly modified form is reproduced on page 13. Your instructor may have you sign it or sign his/her own version of it.

References

Barkley, W. Emmett and John H. Richardson. Chap. 29 in *Methods for General and Molecular Bacteriology*, Philipp Gerhardt, R. G. E. Murray, Willis A. Wood, and Noel R. Krieg, eds. Washington, DC: American Society for Microbiology, 1994.

Collins, C. H., Patricia M. Lyne, and J. M. Grange. Chaps. 1 and 4 in *Collins and Lyne's Microbiological Methods*, 7th ed. Oxford, UK: Butterworth-Heinneman, 1995.

Darlow, H. M. Chap. VI in *Methods in Microbiology,* Volume 1, J. R. Norris and D. W. Ribbins, eds. London, UK: Academic Press, Ltd., 1969.

Emmert, Elizabeth A. B., Jeffrey Byrd, Ruth A. Gyure, Diane Hartman, and Amy White et al. "Biosafety Guidelines for Handling Microorganisms in the Teaching Laboratory: Development and Rationale." *Journal of Microbiology & Biology Education* (May 2013): 78–83. DOI: http://dx.doi.org/10.1128/jmbe.v14i1.531.

Emmert, Elizabeth A. B., Jeffrey Byrd, Ruth A. Gyure, Diane Hartman, and Amy White et al. *Appendix to the Guidelines for Biosafety in Teaching Laboratories*. Washington, DC: American Society for Microbiology, 2012.

Fleming, Diane O. and Debra L. Hunt, eds. *Laboratory Safety— Principles and Practices*, 3rd ed. Washington, DC: American Society for Microbiology, 2000.

Koneman, Elmer W., Stephen D. Allen, William M. Janda, Paul C. Schreckenberger, and Washington C. Winn, Jr. *Color Atlas and Textbook of Diagnostic Microbiology*, 5th ed. Philadelphia and New York: Lippincott-Raven Publishers, 1997.

Power, David A. and Peggy J. McCuen. Pages 2–3 in *Manual of BBL™ Products and Laboratory Procedures*, 6th ed. Cockeysville, MD: Becton Dickinson Microbiology Systems, 1988.

Wilson, Deborah E. and L. Casey Chosewood. U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories,* 5th ed. Washington, DC: U.S. Government Printing Office, 2007.

A Word About Experimental Design

Like most sciences, microbiology has descriptive and experimental components. Here we are concerned with the latter. Science is a philosophical approach to finding answers to questions. Despite what you may have been taught in grade school about *THE* "Scientific Method," science can approach problems in many ways, rather than in any *single* way. The nature of the problem, personality of the scientist, intellectual environment at the time, and good, old-fashioned luck all play a role in determining which approach is taken. Nevertheless, in experimental science, one component that is always present is a **control** (or controls).

A controlled experiment is one in which all **variables** except one—the **experimental variable**—are maintained without change. Frequently, we are looking for the cause of some phenomenon. By limiting variables to only one we can draw a provisional conclusion about whether or not that variable causes the phenomenon.

If changing the variable causes a change in the phenomenon, then we can provisionally conclude that the variable and the phenomenon are causally related; that is, we have demonstrated a **cause and effect relationship** between the phenomenon and the experimental variable. Alternatively, if there is no observed change, we can eliminate the experimental variable from involvement with the phenomenon.

For example, if we want to determine the effect of increasing temperature on microbial growth rate, we could grow the same microorganism in two test tubes containing the same nutrient source at two different temperatures. One would be grown at "normal" temperature (whatever it is for that organism) and the other would be grown at

a higher temperature. Everything but temperature would be the same, so that if we see a difference in growth rate we can attribute it to the experimental variable temperature.

But how would we know there is a difference in growth rate? Simple—by comparing the growth rate at the higher temperature to the growth rate at the "normal" temperature. The "normal" temperature tube is the control; it provides a baseline growth rate against which the experimental growth rate is compared. Growth rate in the experimental tube will be faster, slower, or the same as the control.

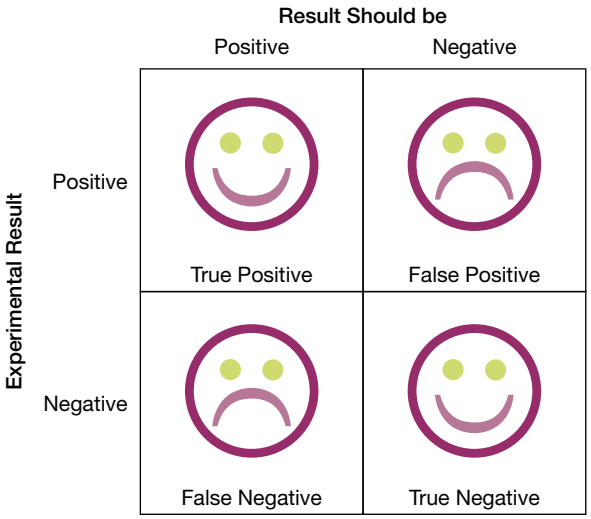
Without the control we would only be able to measure growth rate in the experimental tube, but we couldn't answer the question about the effect of higher temperature on growth rate. Had we grown different microorganisms with different nutrient sources at different temperatures and we saw a difference, we wouldn't know if the difference was due to the organism, the nutrients available, and/or the temperature. This is why we test one variable at a time.

Controls are an essential and integral part of all experiments, but there are many types. Two commonly used controls are **positive** and **negative controls.** A positive control is one that is set up to produce a positive result. Alternatively, a negative control is set up to demonstrate a negative result or no change.

As you work your way through the exercises in this book, pay attention to the various ways positive, negative, and other controls are used to improve the **validity** (effectiveness) of the experiment and the **reliability** (accuracy) of the results. In fact, there are many questions on the data sheets that ask some form of the question: "What is the role of the control in this experiment?" We are pretty sure that your professor won't be satisfied with the answer, "It's a control."

Microbiological experimentation often involves tests that determine the ability of an organism to use or produce some chemical, or to determine the presence or absence of a specific organism in a sample. Ideally, a positive result in the test indicates that the microbe has the ability or is present in the sample, and a negative result indicates a lack of that ability or absence in the sample (Fig. I.9).

The tests we run, however, have limitations and occasionally may give **false-positive** or **false-negative** results. An inability to detect small amounts of the chemical or organism in question would yield a false negative result and would be the result of inadequate **sensitivity** of the test (Fig. I.9). An inability to discriminate between the



I.9 Limitations of Experimental Tests ■ Ideally, tests should give a positive result for specimens that are positive, and a negative result for specimens that are negative. False positive and false negative results do occur, however, and these are attributed to inadequate specificity and inadequate sensitivity, respectively, of the test system.

chemical or organism in question and similar chemicals or organisms would yield false-positive results when the similar chemical or organism was being tested and would be the result of inadequate **specificity** of the test (Fig. I.9). Sensitivity and specificity can be quantified using the following equations:

$$\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}}$$

$$\text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}}$$

The closer sensitivity and specificity are to a value of one, the more useful the test. As you perform the tests in this book, be mindful of each test's limitations, and be open to the possibility of false positive and false negative results.

References

Forbes, Betty A., Daniel F. Sahm, and Alice. S. Weissfeld. Chap. 5 in *Bailey & Scott's Diagnostic Microbiology*, 10th ed. St. Louis, MO: Mosby-Year Book, 1998.

Lilienfeld, David E. and Paul D. Stolley. Page 118 in *Foundations of Epidemiology*, 3rd ed. New York: Oxford University Press, 1994.

Mausner, Judith S. and Shira Kramer. Pages 217–220 in *Epidemiology: An Introductory Text*, 2nd ed. Philadelphia: W.B. Saunders Company, 1985.

Data Presentation: Tables and Graphs

In microbiology, we perform experiments and collect data, but it is often difficult to know what the data mean without some method of organization. Tables and graphs allow us to summarize data in a way that makes interpretation easier.

Tables

A table is often used as a preliminary means of organizing data. As an example, Table I-2 shows the winning times for each male and female age division in a half-marathon race. Again, the aim of a table is to provide information to the reader. Notice the meaningful title, the column labels, and the appropriate measurement units. Without these, the reader cannot completely understand the table and your work will go unappreciated! Data tables are provided for you on the data sheets for each exercise in this book, but you may be required to fill-in certain components (units, labels, etc.) in addition to the data.

TABLE **I-2** **Winning Half-Marathon Times by Sex and Age Division**

Male Runners		Female Runners	
Winner's Age (years)	**Winning Time** (minutes)	**Winner's Age** (years)	**Winning Time** (minutes)
15	73	15	88
25	67	24	82
31	67	30	82
35	71	39	84
40	71	42	85
52	78	50	109
62	95	62	108
70	123	70	126

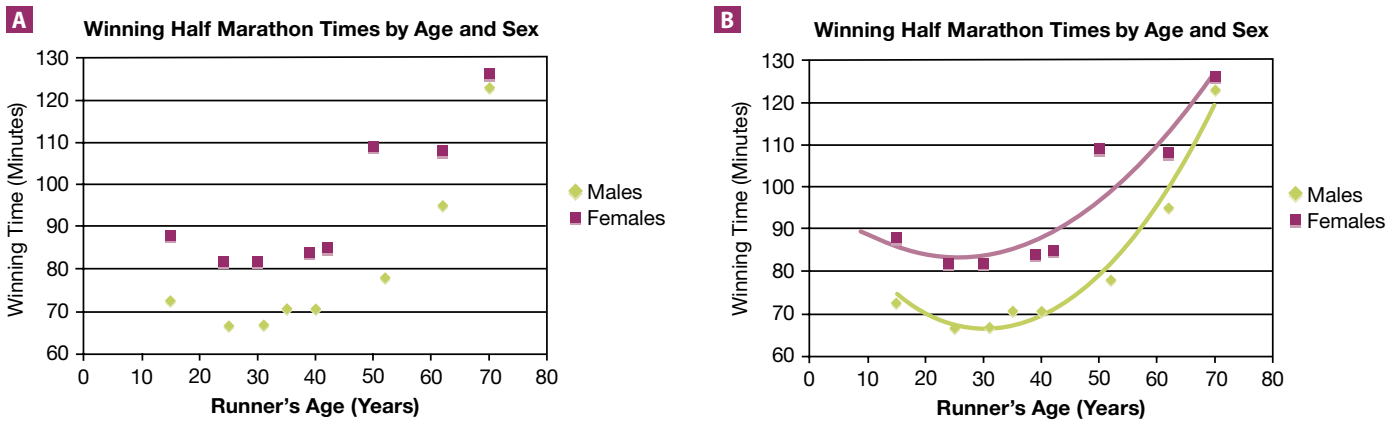
Graphs

Table I-2 does give the information, but what it is telling us may not be entirely clear. It appears that the half-marathon times increase as the runners get older, but we have difficulty determining if this is truly a pattern. That is why data also are presented in graphic form at times; a graph usually shows the relationship between variables better than a table of numbers.

X–Y Scatter Plot The type of graph you will be using in this manual is an "*X*–*Y* Scatter Plot," in which two variables are graphed against each other. Figure I.10 shows the same data as Table I-2, but in an *X*–*Y* Scatter Plot form.

Notice the following important features of the graph in Figure I.10:

- *Title:* The graph has a meaningful title—which should tell the reader what the graph is about. A title of "Age vs. Winning Time" is vague and inadequate.
- *Dependent and independent variables:* The graph is read from left to right. In our example, we might say for the male runners, "As runners get older, winning times get longer." *Winning time* depends on *age*, so winning time is the *dependent* variable and age is the *independent* variable. (Age does *not* depend on the winning time!) By convention, the independent variable is plotted on the *x*-axis and the dependent variable is plotted on the *y*-axis. By way of comparison, notice the consequence of plotting age on the *y*-axis and winning time on the *x*-axis: "As runners get slower, they get older"—which doesn't reflect the actual relationship between the variables and worse yet, is nonsense.
- *Axis labels:* Each axis is labeled, including the appropriate units of measure. "Age" without units is meaningless. Does the scale represent months? Years? Centuries?



I.10 **Sample X-Y Scatter Plot** ■ A graph often shows the relationship between variables better than a table of numbers. Examine this sample and identify the essential components of a quality graph (see text). (**A**) Presentation of data without a best-fit line is acceptable if there are not enough data points to justify illustrating a trend. (**B**) Shown here are the same data but with a trend line. Notice that the points do not fall directly on the line but, rather, that the line gives the general trend of the data. "Connecting the dots" is not appropriate.

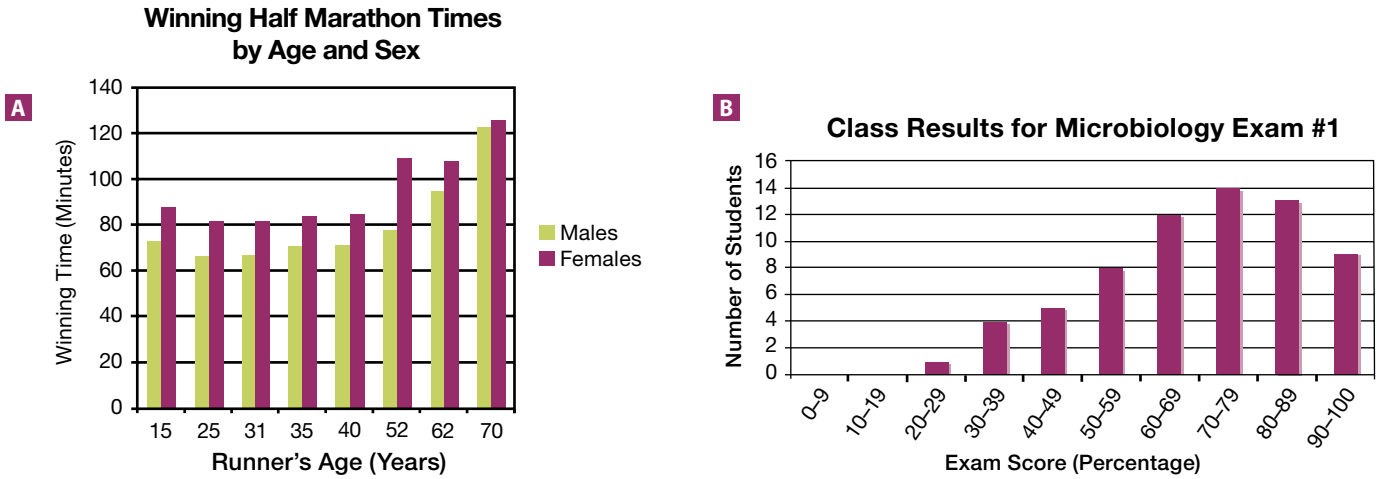
- *Axis scale:* The scale on each axis is uniform. The distance between marks on the axis is always the same and represents the same amount of that variable. (But increments on the *x*-axis don't have to equal those on the *y*-axis, as shown.) The size of each increment is up to the person making the graph and is dictated by the magnitude and range of the data. Most of the time, we choose a length for the axis that fills the available graphing space.
- *Axis range:* The scale for an axis does not have to begin with "0." Use a scale that best presents the data. In this case, the smallest *y*-value was 67 minutes, so the scale begins at 60 minutes.
- *Multiple data sets and the legend:* The two data series (male and female times) are plotted on the same set of axes, but with different symbols that are defined in the legend at the right. The symbols shown differ in color *and* shape, but one of these is adequate.
- *Best-fit line:* If a line is to be drawn at all, it should be an average line for the data points, not one that "connects the dots" (Fig. I.10B). Notice that the points are not necessarily *on* the line. The purpose of a best-fit line is to illustrate the general trend of the data, not the specifics of the individual data points. (Be assured that most graphs in your textbooks where a smooth line is shown were experimentally determined and the lines are derived from points scattered around the line.) There is a mathematical formula that allows one to compute the slope and *y*-intercept of the **trend line** if the relationship is linear, or a **best-fit line** if the relationship is nonlinear (as in the half-marathon times example), but this is beyond our needs. For our purposes, a hand-drawn trend line that looks good is good enough. (If you use a computer graphing program, then it will produce the trend line without you doing any of the math—the best of all situations!)

Bar Graphs

Bar graphs are used to illustrate one variable. Often, the variable is considered to be categorical. That is, the data are in distinct groups (rather than continuous, where the breaks are arbitrary). Using a bar graph to show the relationship between winning times and ages is inappropriate. Examine Figure I.11A. Notice that the space each bar fills is meaningless; that is, the only important part of the bar is the top—which is the value used in the *X*–*Y* scatter plot. Also notice that the *x*-axis scale is not uniform—the gaps between bars range from 4 years (31 to 35) to 12 years (40 to 52). An appropriate use of a bar graph would be the distribution of student performance on an exam, even though exam scores are not categorical (Fig. I.11B). Notice that the space each bar fills has meaning. Each student in a particular group adds height to the bar.

Data Presentation: Be Creative, But Complete

There is no single correct way to produce a graph for a particular data set. Actually, most people working independently would graph the same data set in different ways (e.g., different scales, colors, wording of the title, and axis labels), but the essential components listed would have to be there. You will be asked to graph some of the data you collect. Be sure your graphs tell a complete and clear story of what you have done.



I.11 Bar Graphs ■ A bar graph is appropriate to present data involving a single variable, especially if that variable is easily divided into distinct categories or groups. (**A**) Plotting the winning half-marathon times from Table I-2 using a bar graph is inappropriate because the only meaningful point is at the top. (**B**) A bar graph is useful in presenting data of a single variable, such as the number of students earning a specific score on their microbiology exam.

Student Safety Contract

Student Agreement on Laboratory Safety

I have read the Laboratory Safety Statement as written in *Microbiology: Laboratory Theory & Application, Brief,* 3rd edition, and/or the equivalent document supplied by the Department of Biological Sciences,

_____ ,
(Institution Name)

and I understand its content. I agree to abide by all laboratory rules set forth by the instructor. I understand that my safety is entirely my own responsibility and that I may be putting myself and others in danger if I do not abide by all the rules set forth by the instructor.

COURSE: _____

STUDENT NAME (PRINT): _____

STUDENT SIGNATURE: _____

DATE: _____

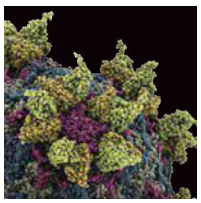
SECTION 1

Fundamental Skills for the Microbiology Laboratory

A necessary skill for safely working in a laboratory, handling foods, and just living in a world full of microbes, is effective hand washing. In Exercises 1-1 and 1-2, you will have the opportunity to evaluate your hand-washing technique to correct any deficiencies you observe and to compare the effectiveness of several hand-cleansing products.

A second skill microbiologists should at the very least be familiar with, and at most be able to do as a matter of routine, is prepare the **media** used to grow bacterial and fungal **cultures**. Media must be sterile prior to use and must also supply the organisms to be grown in or on them with the chemical nutrients necessary for the organisms' growth. Preparation of these media involves weighing ingredients, measuring liquid volumes, calculating proportions, handling basic laboratory glassware, and operating a pH meter and an autoclave. In Exercise 1-3 you will learn and practice these fundamental skills by preparing a couple of simple growth media. When you have completed the exercise, you will have the skills necessary to prepare almost any medium if given the recipe.

A third fundamental skill necessary for any microbiologist is the ability to transfer microbes from one place to another without contaminating the original culture, the new medium, or the environment (including the microbiologist and others in the laboratory). This **aseptic** ("without contamination") transfer technique is required for virtually all procedures in which living microbes are handled, including isolations, staining, and differential testing. Exercises 1-4 through 1-6 present descriptions of common transfer and inoculation methods. Less frequently used methods are covered in Appendices B through D.



A Word About Hand Hygiene

The concept of good hand hygiene has gone from a controversial beginning (in the early 1800s) to an accepted practice that is still problematic. Current studies designed to test the efficacy of various hand-cleansing agents often have subjects wash their hands for unrealistic lengths of time (that is, longer than workers routinely wash on the job), test artificially contaminated hands (or not), and use different standards of evaluation, making comparison difficult. We are still left with the question, "What works best?"

While hand washing has been identified as an important, easily performed behavior that minimizes transfer of pathogens to others, uniform compliance with hand-washing standards has been difficult to achieve. Heavy workloads, skin reactions to the agent (e.g., plain or antimicrobial soap, iodine compounds, alcohol), skin dryness due to frequent washing, and many other factors contribute to noncompliance (Boyce and Pittet, 2002, and The Joint Commission, 2009).

Alcohol-based hand rubs have, in many instances, replaced conventional hand-washing agents because they are more effective than soap and water (disputed), require less time, produce fewer skin reactions, and have been shown to result in a higher level of compliance by health-care workers.

In Exercises 1-1 and 1-2 you will have the opportunity to evaluate your ability to remove artificial "germs" from your own hands and then compare the relative effectiveness of commercial agents used to cleanse hands (or skin in general). ■

Glo Germ™ Hand Wash Education System

Theory

The Glo Germ™ Hand Wash Education System was developed as a training aid for people to learn to wash their hands more effectively. The lotion (a powder is also available) contains minute plastic particles (artificial germs) that fluoresce when illuminated with ultraviolet (UV) radiation but are invisible with normal lighting.

Initially the hands are covered with the lotion, but the location and density of the "germs" is unknown because of the normal room lighting. After washing, a UV lamp is shined on the hands. Wherever the "glowing germs" remain, hand washing was not effective. This provides immediate feedback to the washer as to the effectiveness of their hand washing and provides information about where they need to concentrate their efforts in the future.

Application

Effective hand washing to minimize direct person-to-person and indirect contact transmission of pathogens by health-care professionals and food handlers is essential. It also is critical to laboratorians handling pathogens to minimize transmission to others, inoculation of oneself, and contamination of cultures.

In This Exercise

You will cover your hands with nontoxic synthetic fluorescent "germs" and compare the degree of contamination before and after hand washing to evaluate your hand-washing technique and demonstrate the difficulty in removing hand contaminants.

Materials[1]

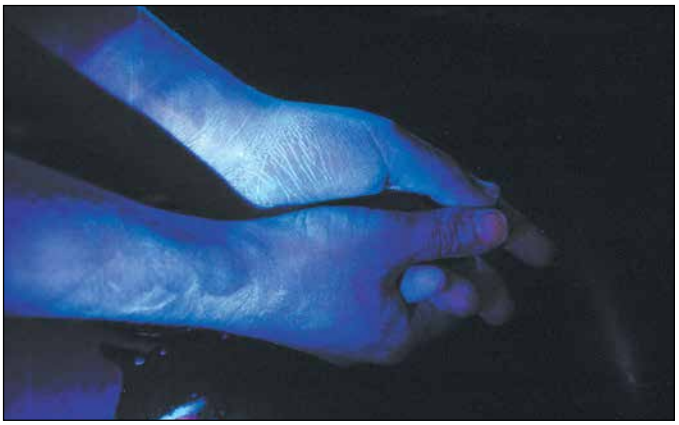
Per Student Group

- ☐ One bottle of Glo Germ™ lotion-based simulated germs
- ☐ One ultraviolet penlight
- ☐ (Optional) fingernail brush

[1] Available from Glo Germ™, 1101 S. Murphy Ln., Moab, UT 84532. 1-800-842-6622 (USA); www.glogerm.com/

PROCEDURE

1. Shake the lotion bottle well.
2. Have your lab partner apply 2–3 drops of gel on the palms of both your hands. Be careful not to get the gel on your clothing, or in your eyes or mouth.
3. Rub your hands together, thoroughly covering your hand surfaces, including the backs and between the fingers (Fig. 1.1). Spread the lotion up to your wrists on both sides. Also, scratch your palms with all fingernails.
4. Have your lab partner shine the UV light on your hands to see the extent of coverage with the lotion. *Do not look directly at the lamp*. This works best in an area with limited ambient light. Do *not* handle the light yourself because you will contaminate it with the artificial germs.
5. Have your lab partner turn on warm water at a sink for you. Then wash your hands with soap and warm water as thoroughly as you can for at least 20 seconds. (If you don't have a watch handy, sing the "Happy Birthday Song" to yourself twice. Or, sing it out loud twice!) Use a fingernail brush if you have one. When you are finished, have your lab partner turn off the water and hand you a fresh paper towel. Dry your hands.
6. Have your lab partner shine the UV light on your hands once more. *Do not look directly at the lamp*. Examine the hand surfaces contaminated by the artificial germs. Then, turn off the lamp.
7. Now that you know where the artificial germs remain, wash your hands once more to remove as many as possible. As before, have your lab partner turn the water on and off for you.
8. Repeat the experiment with your lab partner, but with roles reversed.
9. Record your results on the data sheet on page 19 and answer the questions.
10. After recording your results, shine the UV lamp once more on your data sheet, desk top, and pen/pencil to see how much of the lotion was transferred to these. *Do not look directly at the lamp*.



1.1 Hands Covered With Glo Germ™ Prior to Washing ■ Shown are properly prepared hands covered with the fluorescent Glo Germ™ lotion prior to washing. Note the thorough coverage, including the back of the hands and under the fingernails.

References

Boyce, John M., and Didier Pittet. Centers for Disease Control and Prevention. *Guideline for Hand Hygiene in Health-Care Settings: Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force*. MMWR 51 (No. RR-16) (2002): pages 1–45.

Glo Germ™. Package insert for the Glo Germ™ Hand Wash Education System. www.glogerm.com/

Name _____

Date _____

Lab Section _____

I was present and performed this exercise (initials) _____

DATA SHEET 1-1

Glo Germ™ Hand Wash Education System

OBSERVATIONS AND INTERPRETATIONS

1 Record the degree of hand contamination before and after washing in the table below. Use this qualitative scale for evaluation

+++ means "a lot of contamination"
++ means "moderate contamination"
+ means "little contamination"
0 means "no contamination"

2 There is no absolute cutoff between any of these categories, and what you call "moderate contamination" might be called "little contamination" by another student. Just try to be consistent within your evaluation.

	Left Hand		Right Hand	
Body Region	Before Washing	After Washing	Before Washing	After Washing
Palm				
Fingers				
Between Fingers				
Tops of Fingernails				
Under Fingernails				
Back of Hand				
Front of Wrist				
Back of Wrist				

QUESTIONS

1 *What areas were most thoroughly cleaned by your washing technique?*

2 *What areas were most difficult for you to clean with your washing technique?*

3 *In general, were your two hands cleaned an equal amount, or was one cleaned more than the other? What could account for any differences?*

4 *How do your answers to questions 1, 2, and 3 compare to your lab partner's answers? Why might they differ?*

5 *Why were you instructed to have your lab partner turn the water on and off and operate the UV lamp rather than you doing these actions yourself?*

6 *Why might it be advisable to modify the procedure and use the UV light to check your hands prior to application of lotion and the paper towels prior to drying?*

7 *Using the same qualitative scale as before, record the amount of Glo Germ™ that was transferred to this data sheet, your table top, and to your writing instrument. What does this tell you about the ease of transferring the "unseen" by contact.*

A Comparison of Hand-Cleansing Agents[1]

Theory

Now that you have honed your hand-washing skills in Exercise 1-1, you can compare the relative effectiveness of several hand-cleansing agents. Many hand-cleansing agents are commercially available and each has its appropriate application. Some are intended for routine home use, such as "washing up before dinner." Others are available to the general public, but can be used in clinical settings where prevention of infection is a matter of life and death. And others fall somewhere in-between. Examples of products that can be tested in this exercise are shown in Figure 1.2.

Each product has an **active ingredient** that either kills *(–cidal*, e.g., "bactericidal") or stops the growth of certain microbes *(–static*, e.g., "bacteriostatic"). Their effectiveness comes from an ability to interact with one or more essential cellular components and make them nonfunctional. The cell component could be as small as an enzyme or as large as the entire cytoplasmic membrane, and anything in-between. Thus the active ingredient dictates the utility of the product, because the more damage it does the higher level of decontamination it produces. Question 5 in the data sheet assigns you the task of looking up common active ingredients and reporting on their mechanism(s) of action and their target microbe(s).

[1] Thanks to reviewers Johana Melédez-Santiago and Janice Yoder Smith for their suggestions on developing this laboratory exercise.

Application

Effective hand washing to minimize direct person-to-person transmission, and indirect contact of pathogens by health-care professionals and food handlers, is essential. It also is critical to laboratorians handling pathogens to minimize transmission to others, inoculation of oneself, and contamination of cultures.

In This Exercise

You will evaluate the relative effectiveness of a variety of hand-washing agents.

▼ Materials

Per Student

- □ Lab coat
- □ Disposable gloves
- □ Chemical eye protection
- □ One nutrient agar or tryptic soy agar plate
- □ Access to a sink and paper towels
- □ Permanent marking pen

Per Table

- □ Paper towels
- □ Tap water

1.2 Hand-Washing Agents ■ From left to right are: Betadine® scrub (10% povidone iodine), alcohol-based hand sanitizer (65% ethyl alcohol, 0.13% benzalkonium), chloride-based hand sanitizer, antibacterial soap (0.15% triclosan), and Hibiclens® (4% chlorhexidine gluconate). As written, this exercise uses these agents, but others can be substituted, including tap water.

1

- ☐ Bottles of as many as five of the following or suitable replacements:
  - Antibacterial liquid soap (0.15% triclosan)
  - Hand sanitizer (65% ethyl alcohol = 65% ethanol)
  - Hy5 brand of alcohol-free hand sanitizer (0.13% benzalkonium chloride)
  - Betadine® scrub (10% povidone iodine)
  - Hibiclens® (4% w/v chlorhexidine gluconate)

Note

Students should not ingest or get any of these products in their eyes. Follow package inserts for treatments. Students with allergies or sensitivities to the active ingredients in any of these products should be assigned an alternate product.

PROCEDURE

Lab One

1. Wear a lab coat, gloves, and chemical eye protection when performing this procedure.
2. Label the base of a nutrient agar or tryptic soy agar plate with your name. Divide the plate in half and label one half "Before" and the other half "After." Then, number 1 through 5 on both sides of the dividing line so the numbers line up. That is, number 1 on the "Before" side should be opposite number 1 on the "After" side, and so on. Use the entire diameter of the plate for the five numbers and spread them out as much as you can. You will be pressing your fingers at each number in steps 4 and 7.
3. Rub your left-hand fingertips (all five of them) on the area to be sampled. (Good choices are the floor, shoe soles, tabletop near a sink, backpack, and your forehead or feet. Be creative!)
4. Then, one at a time, gently press your thumb tip next to the "Before" number 1, your index finger next to the "Before" number 2, and so on. Your middle, ring, and pinky fingers are numbered 3, 4, and 5, respectively.
5. Wash your hands with soap and water.
6. Record the hand-cleansing agents you will be using on the data sheet, page 23.
7. Now sample the same site used in step 2 with the fingertips of your right hand, but this time one fingertip at a time. Then, you will rub that fingertip in a few milliliters of the hand-cleansing agent in your left palm (see steps a and b for further directions). If the agent requires rinsing and drying, perform this at a sink. Agents that only require air-drying can be done at your table.
   a. Place a quarter-size drop of the first hand-cleansing agent in the palm of your left hand. Rub your right thumb on the area to be sampled. Then, rub your thumb into the agent for 10 seconds.[2] (If you don't have a watch, sing the "Happy Birthday Song" once to yourself.) Once your thumb is dry, gently press it next to the number 1 on the "After" side. Wash both hands with soap and water, and dry them.
   b. Repeat step a with each finger of the right hand and the remaining four hand-cleansing agents. Be sure to press each finger onto the agar next to its correct number and to wash your hands in between exposures.
8. Tape the lid on the plate and incubate at 25°C until the next lab period.

Lab Two

1. Examine your plates for growth. (***Note:*** do not remove the plate's lid unless given permission to do so.) Each "blob" is probably the product of a single microbial species that has reproduced from one or a few cells to such an extent that it is now visible to the naked eye. This is called a "colony." Different microbial species often produce distinctly different colonies.
2. Fill in the table provided on the data sheet, page 24, as you evaluate the relative amount of growth and microbial diversity (based on colony differences) on the "Before" and "After" sides of the plate for each agent. Recognize that you are comparing "apples and oranges" with respect to their intended uses. That is, the products tested range from surgical scrubs to over-the-counter soaps intended for household use. A surgical scrub may have better antimicrobial activity than hand soap, but that doesn't make it "better" for household use!
3. Dispose of all plates in the appropriate autoclave container when finished.

[2] The recommended time for hand washing is 15–20 seconds, but because you are only washing the fingertips and concentrating on those, that time has been reduced in order to see representative results.

References

Boyce, John M. and Didier Pittet. Centers for Disease Control and Prevention. *Guideline for Hand Hygiene in Health-Care Settings: Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force*. MMWR 51 (No. RR-16) (2002): 1-45.

The Joint Commission, 2009. *Measuring Hand Hygiene Adherence: Overcoming the Challenges*. Available online. URL: http://www.jointcommission.org/assets/1/18/hh_monograph.pdf.

Name _____

Date _____

Lab Section _____

I was present and performed this exercise (initials) _____

DATA SHEET 1-2

A Comparison of Hand-Cleansing Agents

OBSERVATIONS AND INTERPRETATIONS

1 Record your results in the table below. For **relative amount of growth**, use this qualitative scale for evaluation:
+++ means "a lot of contamination"
++ means "moderate contamination"
+ means "little contamination"
0 means "no contamination"

2 There is no absolute cutoff between any of these categories. What you call "moderate contamination" might be called "little contamination" by another student. Just try to be consistent within your evaluation. For **diversity**, look for different colors, shapes, textures, sizes, and anything else that is indicative of a different organism growing, and record the number of different organisms present. **Interpretation** would be relative effectiveness of the agent (excellent, good, fair, poor).

Number on Plate	Hand-washing Agent	"Before"		"After"		Interpretation
		Relative Growth	Diversity	Relative Growth	Diversity	
1						
2						
3						
4						
5						

QUESTIONS

1 *Which agent seemed to be most effective at removing microbes from the fingertips?*

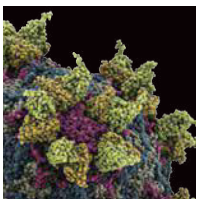
2 *Which agent seemed to be least effective at removing microbes from the fingertips?*

3 *What other variables besides the cleansing agent might be responsible for any differences noted between fingers or students?*

4 *Did you or any other students notice some microbes persisting more than others after washing? What factor(s) might account for this?*

5 *Do some research and fill in the following table. Your answer to "Antibacterial Effect" should address the structural and/or functional part of the cell affected, as well as if the agent is bactericidal (kills bacteria) or bacteriostatic (stops bacterial growth, but doesn't kill them). Under "Other Antimicrobial Effects," note any antiviral, antiprotozoal, and/or antifungal properties of the agent.*

Active Ingredient	Antibacterial Effect	Other Antimicrobial Effects
Triclosan		
Isopropanol/ethanol (isopropyl/ethyl alcohol)		
Benzalkonium chloride		
Povidone iodine scrub		
Chlorhexidine gluconate		



A Word About Basic Growth Media

To cultivate microbes, microbiologists use a variety of growth media. Although these media may be formulated from scratch, they more typically are produced by rehydrating commercially available powdered media. Media that are routinely encountered in the microbiology laboratory range from the widely used, general-purpose growth media, to the more specific selective and differential media used in identification of microbes (Sections 4 and 5). In Exercise 1-3 you will learn how to prepare simple general growth media. ■

EXERCISE 1-3

Nutrient Broth and Nutrient Agar Preparation

■ Theory

Nutrient broth and nutrient agar are common media used for maintaining bacterial cultures[1]. To be of practical use, they have to meet the diverse nutrient requirements of routinely cultivated bacteria. As such, they are formulated from sources that supply carbon and nitrogen in a variety of forms—amino acids, purines, pyrimidines, monosaccharides to polysaccharides, and various lipids.

Generally, these are provided in digests of plant material (phytone) or animal material (peptone and others). Because the exact composition and amounts of carbon and nitrogen in these ingredients are unknown, these media are considered to be **undefined**. They are also known as **complex media**[2].

In most classes (because of limited time), media are prepared by a laboratory technician or by the instructor. Still, it is instructive for novice microbiologists to at least gain exposure to what is involved in media preparation. Your instructor will provide specific instructions on how to execute this exercise using the equipment in your laboratory.

■ Application

Microbiological growth media are prepared to cultivate microbes. These general growth media are used to maintain bacterial stock cultures.

[1] This is a true statement but a bit misleading. The implication is that these will support growth of most bacteria. In fact, recent estimates indicate we have yet to find appropriate media and laboratory conditions to cultivate over 90% of bacteria in the environment!

[2] In contrast, **chemically defined** media are composed of ingredients with a known chemical structure and in known quantities. For an example, see the recipe for Citrate Agar (Simmons) on page 612. In it, the only carbon source is the six-carbon sugar citrate ($C_6H_8O_7$) and the only nitrogen source is ammonium (NH_4^+).

■ In This Exercise

You will prepare 1-liter batches of two general growth media: nutrient broth and nutrient agar. During the course of the semester, a laboratory technician will probably do this for you, but it is good to gain firsthand appreciation for the work done behind the scenes!

▼ Materials

Per Student

- □ Lab coat
- □ Disposable gloves
- □ Chemical eye protection

Per Student Group

- □ One 2-liter Erlenmeyer flask for each medium made
- □ Three or four 500 mL Erlenmeyer flasks and covers (can be aluminum foil)
- □ Three or four stirring hot plates
- □ Three or four magnetic stir bars
- □ All ingredients listed in the following recipes (or commercially prepared dehydrated media)
- □ Sterile Petri dishes
- □ Test tubes (16 mm × 150 mm) and caps
- □ Balance
- □ Weighing paper or boats
- □ Spatulas

■ Medium Recipes

Nutrient Broth

□ Beef extract	3.0 g
□ Peptone	5.0 g
□ Distilled or deionized water	1.0 L

pH 6.6–7.0 at 25°C

1

Nutrient Agar

□ Beef extract	3.0 g
□ Peptone	5.0 g
□ Agar	15.0 g
□ Distilled or deionized water	1.0 L
pH 6.6–7.0 at 25°C	

Note that the only difference between nutrient broth and nutrient agar is the agar. Agar is indigestible by most bacteria so it is not considered a nutrient source in the medium. It acts solely as a solidifying agent in which to suspend the nutrients, which makes the name "nutrient agar" a bit misleading. Also note the agar concentration: 15 g/L is 15 g/1,000 mL = 1.5 g%. You will notice different agar concentrations in other media you encounter during the course.

PREPARATION OF THE MEDIA

Lab One

To minimize contamination while preparing media clean the work surface, turn off all fans, and close any doors that might allow excessive air movements. Wear a lab coat, gloves, and chemical eye protection when performing these procedures.

Note

Your instructor will determine how many tubes and plates of each medium individual students will prepare. Recipes are given in 1 L volumes and will be enough to prepare approximately 140 tubes at 7 mL and 50 plates at 20 mL. Thus, in a class of 24 students 1 L of broth and 1 L of agar would permit each student to prepare 5 or 6 tubes and 2 plates, respectively. Smaller batches can be made if greater emphasis is placed on more students experiencing the weighing and mixing aspect of medium preparation. For example, pairs of students in a class of 24 could prepare 100 mL batches, which would allow each student to make approximately 7 tubes or 2 to 3 plates.

Nutrient Agar Tubes

1. Weigh the ingredients on a balance (Fig. 1.3).
2. Suspend the ingredients in 1 L of distilled or deionized water in the 2-liter flask, mix well, and boil until fully dissolved (Fig. 1.4).
3. Dispense 7 mL portions into test tubes and cap loosely (Fig. 1.5). If your tubes are smaller than those listed in Materials, adjust the volume to fill 20% to 25% of the tube. Fill to approximately 50% for agar deeps.
4. Sterilize the medium by autoclaving for 15 minutes at 121°C (Fig. 1.6).
5. After autoclaving, cool to room temperature with the tubes in an upright position for agar deep tubes. Cool with the tubes on an angle for agar slants (Fig. 1.7).
6. Incubate the slants and/or deep tubes at 35 ± 2°C for 24 to 48 hours.

1.3 Weighing Medium Ingredients ■ Solid ingredients are weighed with an analytical balance. A spatula is used to transfer the powder to a tared weighing boat. Shown here is dehydrated nutrient agar, but the weighing process is the same for any powdered ingredient.

1.4 Mixing the Medium ■ The powder is added to a flask of distilled or deionized water on a hot plate. A magnetic stir bar mixes the medium as it is heated to dissolve the powder.

Nutrient Agar Plates

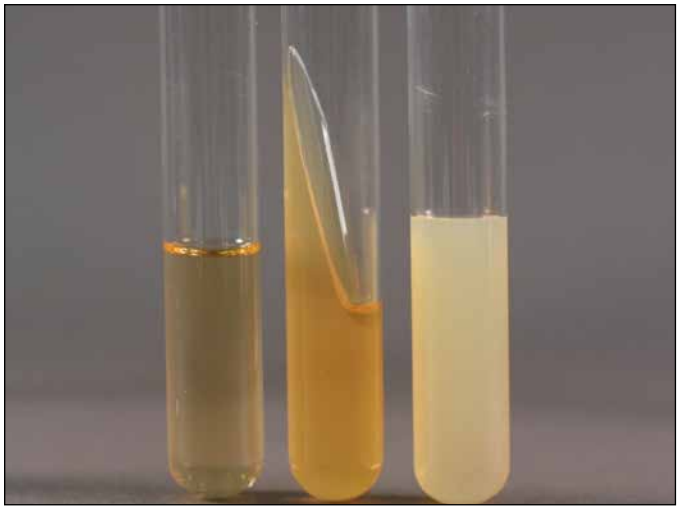
1. Weigh the ingredients on a balance (Fig. 1.3).
2. Suspend the ingredients in 1 L of distilled or deionized water in the 2-liter flask, mix well, and boil until fully dissolved (Fig. 1.4).
3. Divide into three or four 500 mL flasks for pouring. Smaller flasks are easier to handle when pouring plates. Don't forget to add a magnetic stir bar and to cover each flask before autoclaving.
4. Autoclave for 15 minutes at 121°C to sterilize the medium.
5. Remove the sterile agar flasks from the autoclave and allow them to cool to 50°C while stirring on a hot plate.
6. Dispense approximately 20 mL into sterile Petri plates (Fig. 1.8). **Be careful! The flask will still be hot, so wear an oven mitt.** While you pour the agar, shield the Petri dish with its lid to reduce the chance of introducing airborne contaminants. If necessary, *gently* swirl each plate so the agar completely covers the bottom; do not swirl the agar up into the lid. Allow the agar to cool and solidify before moving the plates (Fig. 1.9).
7. Store these plates on a countertop for 24 hours to allow them to dry prior to use.

Nutrient Broth

1. Weigh the ingredients on a balance (Fig. 1.3).
2. Suspend the ingredients in 1 L of distilled or deionized water in the 2-liter flask. Agitate and heat slightly (if necessary) to dissolve them completely (Fig. 1.4).
3. Dispense 7 mL portions into test tubes (or less, depending on your lab customs) and cap loosely (Fig. 1.5). As with agar slants, if your tubes are smaller than those recommended in Materials, add enough broth to fill them approximately 20% to 25%.
4. Sterilize the medium by autoclaving for 15 minutes at 121°C (Fig. 1.6).

1.6 Autoclaving the Tubed Media ■ Media are sterilized in an autoclave for 15 minutes at 121°C. Shown are four racks of tubed media being removed from an autoclave. The tubes on the left are brain-heart infusion broth (BHI or BHIB) and they will be allowed to stand and cool. Once cool, they will remain liquid. The tubes on the right contain brain-heart infusion agar (BHIA), a solid medium, which is liquid when it is removed from the autoclave but will solidify as it cools due to the agar in it. The position of the tube as it cools will determine whether an agar deep tube or an agar slant is produced (Fig. 1.7). Note the angled sides of the racks that allow them to be tipped so the agar will solidify in a slant. Plated media are autoclaved in a flask and then dispensed into sterile Petri dishes (Fig. 1.8).

1.5 Dispensing the Medium into Tubes ■ An adjustable pump can be used to dispense the appropriate volume (usually 7 mL–10 mL) into tubes. Then, loosely cap the tubes.



1.7 Tubed Media ■ From left to right: a broth, an agar slant, and an agar deep tube. The solid media are liquid when they are removed from the autoclave. Agar deeps are allowed to cool and solidify in an upright position, whereas agar slants are cooled and solidified on an angle.

Lab Two

1. Examine the tubes and plates for evidence of growth.
2. Record your observations on the data sheet, page 29.
3. Save the plates and tubes as directed by your instructor.

1.8 Pouring Agar Plates ■ Agar plates are made by pouring sterilized medium into sterile Petri dishes. The lid is used as a shield to prevent airborne contamination. Once poured, the dish is gently swirled so the medium covers the base. Plates are then cooled and dried to eliminate condensation.

1.9 An Agar Plate ■ Plated media are often used for isolating individual species from a mixed culture (Exercises 1-5 and 1-6) or for counting the number of cells in a diluted sample (Exercise 6-2). Some differential tests also use plated media (e.g., milk agar, DNase agar, starch agar). Shown is a BHIA plate, which stands for "brain-heart infusion agar." The 1.5%–2% agar in the medium acts as a solidifying agent to suspend the nutrients—extracts of brain and heart tissues. Brain and heart provide carbon, nitrogen, and other essential nutrients for growth, as well as energy.

Reference

Zimbro, Mary Jo and David A. Power. Pages 404–405 and 408 in *DIFCO™ & BBL™ Manual—Manual of Microbiological Culture Media*. Sparks, MD: Becton Dickinson and Company, 2003.

Name _____

Date _____

Lab Section _____

I was present and performed this exercise (initials) _____

DATA SHEET 1-3

Nutrient Broth and Nutrient Agar Preparations

OBSERVATIONS AND INTERPRETATIONS

1 In the following table, record the number of each medium you prepared, and then record the number of apparently sterile ones. Calculate your percentage of successful preparations for each. In the last column, speculate as to probable/possible sources of contamination (even if all your media are sterile).

Medium	Total Number Prepared	Number of Sterile Preparations	Percentage of Successful Preparations	Probable Sources of Contamination
Nutrient agar tubes (slant or deep)				
Nutrient agar plates				
Nutrient broths				

QUESTIONS

1 *Which medium was most difficult to prepare without contamination? Why do think this might be so?*

1

2 *For each of the following types of contamination suggest the most likely point in preparation (or later) at which the contaminant was introduced.*

a. *Growth in all broth tubes.*

b. *Growth in one broth tube.*

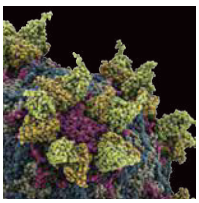
c. *Growth only on the surface of a plate.*

d. *Growth throughout the agar's thickness on a plate.*

e. *Growth only in the upper 1 cm of agar in an agar deep tube.*

f. *All plates in a batch have the same type and density of contaminants.*

g. *Only a few plates in a batch are contaminated, and each looks different.*

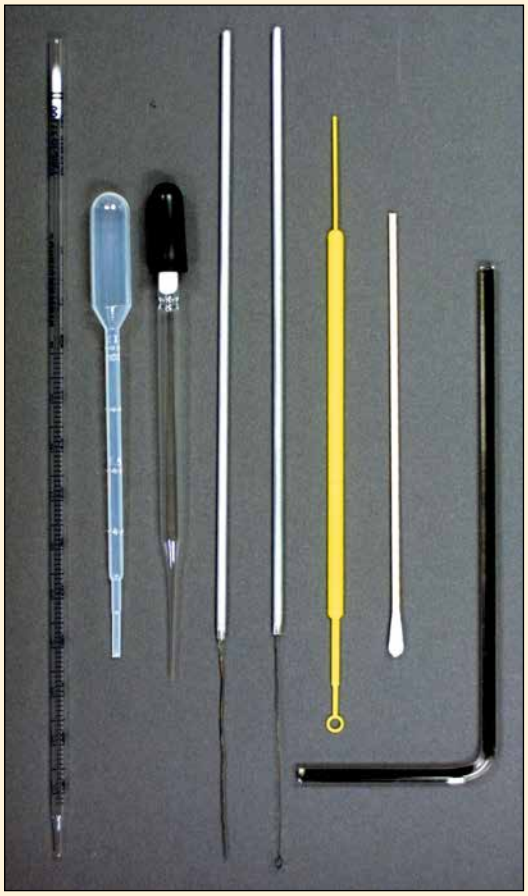


A Word About Aseptic Transfers and Inoculation Methods

Microbiologists must be able to transfer living microbes from one place to another aseptically (i.e., without contamination of the culture, the sterile medium, or the surroundings). Microbiology students must also acquire this skill and do so very early on. While you won't be expected to master all transfer methods right now, you will be expected to perform most of them over the course of the semester. Refer to Exercises 1-4 through 1-6 and Appendices B, C, and D as needed.

To prevent contamination of the sample, inoculating instruments (Fig. 1.10) must be sterilized prior to use. Wire inoculating loops and needles are sterilized immediately before use in an incinerator or Bunsen burner flame. The mouths of tubes or flasks containing cultures or media are also incinerated at the time of transfer by passing their openings through a flame. Instruments that are not conveniently or safely incinerated, such as Pasteur pipettes, cotton applicators, glass pipettes, and digital pipettor tips, are sterilized inside wrappers or containers by autoclaving prior to use.

Aseptic transfers are not difficult; however, a little preparation will help assure a safe and successful procedure. Before you begin, you will need to know where the sample is coming from, its destination, and the type of transfer instrument to be used. These exercises provide step-by-step descriptions of routine transfer methods. Certain less-routine transfer methods are discussed in Appendices B through D. ■



1.10 Inoculating Instruments ■ Any of several different instruments may be used to transfer a microbial sample, the choice of which depends on the sample source, its destination, and any special requirements imposed by the specific protocol. Shown here are several examples of transfer instruments. From left to right: serological pipette (see Appendix C), disposable transfer pipette, Pasteur pipette, inoculating needle, inoculating loop, disposable inoculating needle/loop, cotton swab (see Appendix B and Exercise 1-5), and glass spreading rod (see Exercise 1-6). (***Note:*** the glass rod is not an inoculation instrument, but it is used to spread an inoculum introduced to an agar plate by another instrument. As such, it is an instrument used in an inoculation process.) When transferring BSL-2 organisms, we advise using a sterile disposable loop or wooden stick (not shown). Neither of these requires incineration after use and each minimizes the threat of aerosol production.

Common Aseptic Transfers and Inoculation Methods

EXERCISE 1-4

■ Theory

A medium that contains living microbes is called a **culture**. If a culture contains a single species it is said to be a **pure culture**. It is essential to transfer microbes from their pure culture to a sterile medium **aseptically**, that is, without contamination of yourself, others, the environment, the source culture, or the medium being inoculated. In other words, you want your pure culture to stay pure, your new culture to be pure, and the surroundings to remain uninoculated.

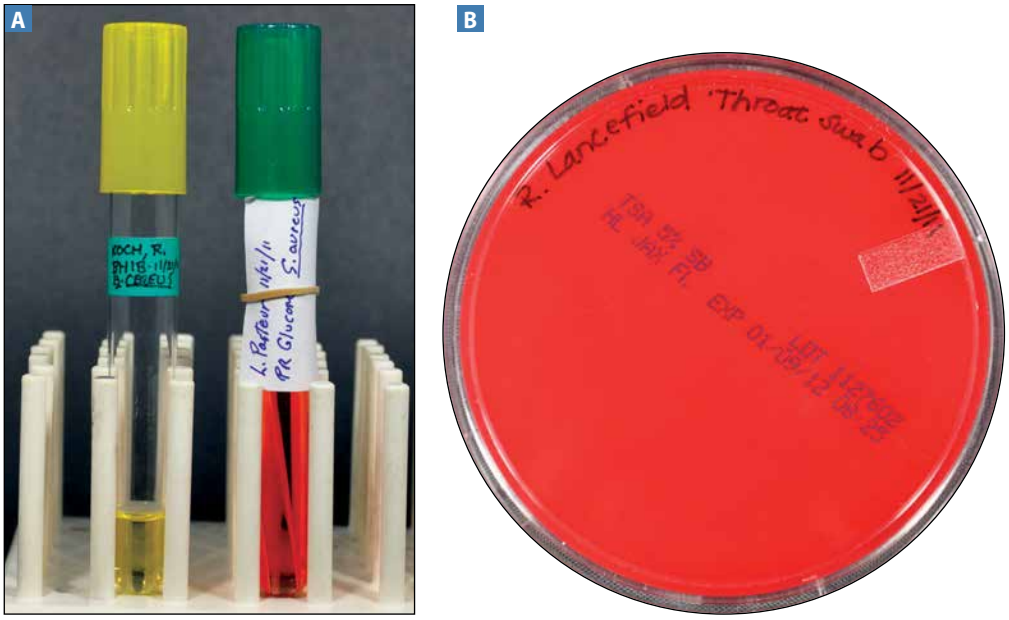
General Techniques and Practices for Aseptic Transfers

Following are general techniques and practices that improve your chances of successfully making an aseptic transfer. Equally important are the techniques related to safety. It has been demonstrated that while "…the causative incident for most LAIs (laboratory acquired infections) is unknown…A procedure's potential to release microorganisms into the air as aerosols and droplets is the most important operational risk factor…" (Chosewood and Wilson, 2009). In other words, limiting

aerosol production is a *safety issue and not an issue of keeping pure cultures pure*. Please adhere to these practices for your safety and the safety of those around you.

- **Minimize the potential of contamination.** Do not perform any transfers over your books and papers because you may inadvertently and unknowingly contaminate them with droplets or aerosols that settle. Put them safely away. Some labs advise performing transfers over a disinfectant-soaked paper towel.
- **Be organized.** Arrange all media in advance and clearly label them with your name, the date, the medium, and the inoculum (Fig. 1.11). Tubes may be labeled with tape, paper held on with a rubber band, or by writing directly on the glass (this option presumes you will clean the writing off before you dispose of the culture for decontamination). You *should* write directly on the base (not the lid) of plastic Petri plates because they are disposable. Be sure not to place any labels in such a way as to obscure or obstruct your view of the tube's or plate's interior.
- **Place all media tubes in a test tube rack when not in use whether they are sterile or not.** Tubes should never be laid on the table surface because they may leak (Fig. 1.12).
- **Take your time.** Work efficiently, but do not hurry. You are handling potentially dangerous microbes. Working at a frenzied pace leads to carelessness and accidents.
- **Never hold a tube culture by its cap.** Caps are generally loose to allow aeration and are not secure enough to be used as a handle. Even screw caps can be loose enough so as not to be secure.
- **Hold the inoculating loop or needle like a pencil in your dominant hand and relax** (Fig. 1.13)!
- **Adjust your Bunsen burner so its flame has an inner and outer cone** (Fig. 1.14).

1.12 Microbiologist at Work ■ Materials are neatly positioned and not in the way, and the Bunsen burner is accessible, but not so close as to be a major fire hazard. To prevent spills, culture tubes are stored upright in a test tube rack. They are never laid on the table. The microbiologist is relaxed and ready for work. Notice he is holding the loop like a pencil, not gripping it like a dagger.



1.11 Label the Media ■ To avoid confusion after-the-fact, it is best to label sterile media prior to inoculating it. (A) Tubed media can be labeled with tape, paper labels, or directly on the glass with a marking pen. Labels must be removed when tubes are put in the autoclave bin for sterilization. (B) Plastic Petri dishes should be labeled on their base, not on their lid, because the lid may get separated from its base during reading or rotated from its correct orientation. Write the information at the edge to avoid obscuring growth on the plate. Because most labs use disposable Petri dishes, the labels do not have to be removed prior to autoclaving.

1.13 Hold the Loop like a Pencil ■ Holding the loop as shown puts the hand in a convenient position to hold tube caps with the "pinky" finger.

1.14 Bunsen Burner Flame ■ When properly adjusted, a Bunsen burner produces a flame with two cones. Sterilization of inoculating instruments is done in the hottest part of the flame—the tip of the inner cone (red arrow). Heat-fixing bacterial smears on slides and incinerating the mouths of open glassware items are done in the outer cone (white arrow).

Types of Media

Media come in many forms, each with specific applications. **Broths** are used to grow microbes when fresh cultures or large numbers of cells are required. Broths of differential media are also used in microbial identification (Section 5). **Agar slants** are generally used to grow stock cultures that can be refrigerated after incubation and maintained for several weeks. In addition, many differential media are agar slants. **Plated media** are typically used for obtaining isolation of species (Exercises 1-5 and 1-6), differential testing, and quantifying bacterial densities (Exercise 6-1). In all cases, using these media requires aseptic inoculation in which a portion of an existing pure culture is transferred to a sterile medium to start a new pure culture. (A streak plate and sometimes a spread plate are exceptions to this. See Exercises 1-5 and 1-6.)

Transfers can be made between all forms of media—slants, broths, and plates—depending on the intended use of the new culture. The following is organized into transfers from broth culture to sterile broth, agar slant culture to sterile agar slant, and plate culture to sterile broth. If you can do these, then you have the skills to transfer between most any combination of media. (Inoculation of sterile agar plates is covered in Exercises 1-5 and 1-6 and will complete your skill set.)

Transfer Instruments

The instruments usually used for transfers are either **inoculating loops** or **inoculating needles**. (Pipettes are also sometimes used and these are covered in Appendices C and D.) For simplicity, the following instructions only refer to inoculating loops, but the same apply to inoculating needles.

A Special Note about Transferring BSL-2 Organisms

Most college biology teaching laboratories have eliminated or reduced the use of BSL-2 organisms, as we have in this edition of the lab manual. However, use of some BSL-2 organisms is unavoidable for some tests. In other exercises, they are included as optional test organisms.

The primary concern with BSL-2 organisms is aerosol production, which can lead to contamination of the environment or infection due to inhalation. Aerosols are problematic because we generally are unaware of their production and they remain suspended in the air long after the procedure has been completed.

Throughout this section we emphasize techniques that minimize aerosol production, but they are even more essential when handling BSL-2 organisms. Your lab will have specific guidelines on how to handle BSL-2 organisms and you should take these seriously. They may include any or all of the following precautions, depending on the exercise and the equipment you have available.

- Performing tests in a Class I or II biosafety cabinet (Fig. I.1) is recommended, but not all teaching laboratories have these installed.
- Using an electric incinerator (Fig. 1.15) for decontaminating wire loops and needles, which simultaneously contains aerosols within the ceramic interior and decontaminates them.
- Using sterile disposable loops/needles or wooden sticks that don't require flaming after use and can be disposed of in sharps containers or other appropriate receptacles for autoclaving.
- Wearing gloves and eye protection.
- Identifying BSL-2 organisms with red caps on tube cultures or BSL-2 labels on plate cultures.

Whatever precautions your college's guidelines dictate, take them seriously. Your health and the health of others in your lab are at stake.

Transfer from a Broth Culture to a Sterile Broth

As you read these instructions, also follow the procedural diagram in Figure 1.16 to get a summary view of the process. **Make appropriate adjustments if handling a BSL-2 organism.**

1. Label the sterile broth tube with your name, the date, the medium, and the organism you are inoculating it with.
2. Make sure your loop is a closed circle. If it isn't, pinch it closed. Hold it like a pencil, and then flame it from base to tip as shown in Figure 1.17. Be sure the entire wire becomes orange-hot at some point.
3. Suspend the bacteria in the broth culture with a vortex mixer prior to transfer (Fig. 1.18). Be sure not to mix so vigorously that broth gets into the cap or that you lose control of the tube. Start slowly, and then gently increase the speed until the tip of the vortex reaches the bottom of the tube. Alternatively, the culture may be agitated by drumming your fingers along the length of the tube several times (Fig. 1.19). Again, be careful not to splash broth into the cap or lose control of the tube.
4. Loosen the cap of the culture tube (this is especially important if you are using a screw-cap tube). Move the culture tube to your loop hand. Remove and hold the tube's cap with the little finger of your loop hand (Fig. 1.20). Moving the tube and not the loop prevents excessive movement of the loop that might result in aerosols or droplets.
5. Incinerate the lip of the tube by passing it quickly through the flame two or three times (Fig. 1.21). Do not wave it through the flame so fast that broth sloshes out of the open end.
6. Hold the tube on an angle to minimize the opportunity for airborne contamination (Fig. 1.22).
7. This step is important to minimize the production of aerosols. Hold the loop hand still and move the tube up the wire until the loop's tip is in the broth. Continue holding the loop hand still while you remove the tube from over the loop (Fig. 1.23). *Be careful not to catch the loop on the lip of the tube or you will produce contaminated droplets and aerosols*. At this point, there should be a visible film of broth in your loop (Fig. 1.24). If there is not a visible film of broth, replace the cap, flame the loop, let it cool, and then pinch the loop so it is a closed circle. Then, start over.
8. While holding your loop hand still, flame the tube and replace the cap. Set the tube in the rack and pick up the sterile broth tube in your free hand.
9. Repeat the process of removing the cap with the little finger of the loop hand and flaming the lip of the sterile broth tube while holding the loop hand still. Hold the tube on an angle. *Don't forget you have living microbes exposed to the environment on the loop at this point and careless movements can spread them.*
10. Move the tube over the loop until it is submerged in the sterile broth and mix by gently swirling the loop.
11. Before you remove the loop from the tube, tap the loop's face on the inside of the tube to remove the

1.15 Bacteriological Incinerator ■ Bacterial incinerators use infrared heat and reach temperatures over 800°C. A wire loop/needle is inserted into the incinerator and heated for 5–7 seconds. (The handle may also get hot, so be careful. You may wish to wrap the handle in several layers of tape for insulation.) The loop/needle is then removed and allowed to cool without touching anything. It may then be used to transfer microbes, or if this is done at the completion of a transfer, it may be set-aside in a holder (often in the base of the incinerator, as shown) until needed again.

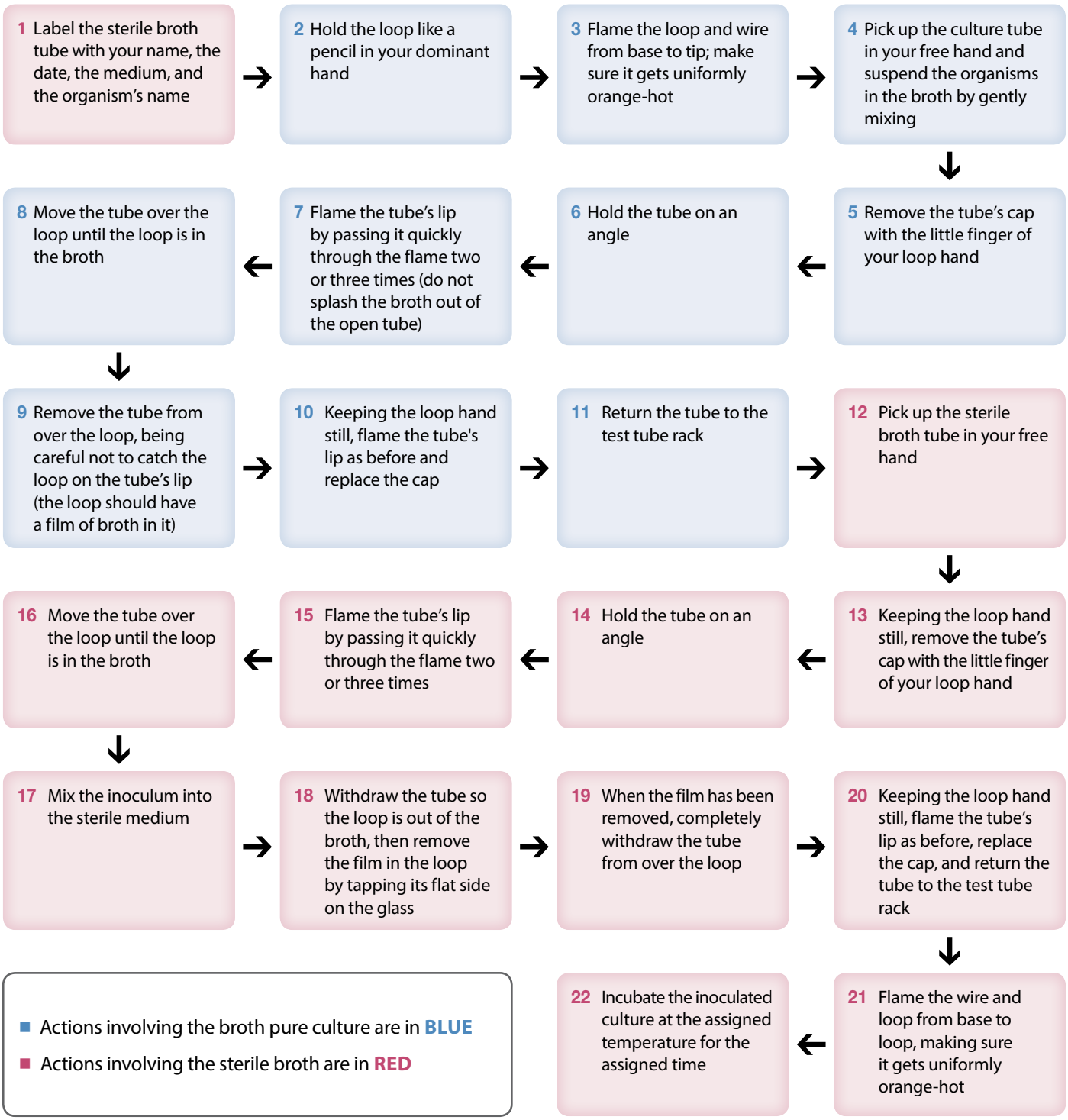
film within the loop (Fig. 1.25). Be persistent in this—don't give up until you are successful at its removal. If you were to flame the loop with the film present, you would produce aerosols.

12 Carefully remove the tube from over the loop and avoid catching the loop on the lip of the tube.

13 Keeping your loop hand still, flame the tube, replace the cap, and set it in the rack.

14 Flame the loop from base to tip until it is uniformly orange-hot.

15 Incubate the inoculated culture at the assigned temperature for the assigned time.



1.16 Procedural Diagram: Aseptic Transfer from a Broth Pure Culture to a Sterile Broth Tube ■ This is a summary of the procedure. Make every effort to keep your loop hand as still as possible throughout the transfer. Details can be found in the text. Make appropriate adjustments if transferring a BSL-2 organism.

1.17 Flaming the Loop ■ Incineration of an inoculating loop's wire is done by passing it through the tip of the flame's inner cone. Begin at the wire's base and continue to the end, making sure that all parts are heated to a uniform orange color. Allow the wire to cool before touching it or placing it on/in a culture. The former will burn you; the latter will cause aerosols of microorganisms.

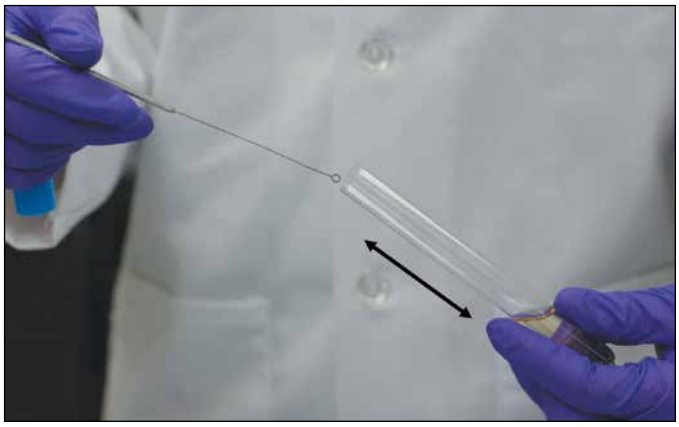
1.19 Mixing Broth by Hand ■ A broth culture always should be mixed prior to transfer. Tapping the tube with your fingers gets the job done safely and without special equipment.

1.21 Flaming the Tube ■ The tube's mouth is passed quickly through the flame a couple of times to sterilize the tube's lip and the surrounding air. Do not move the tube so quickly that broth sloshes out the opening. Notice that the tube's cap is held in the loop hand.

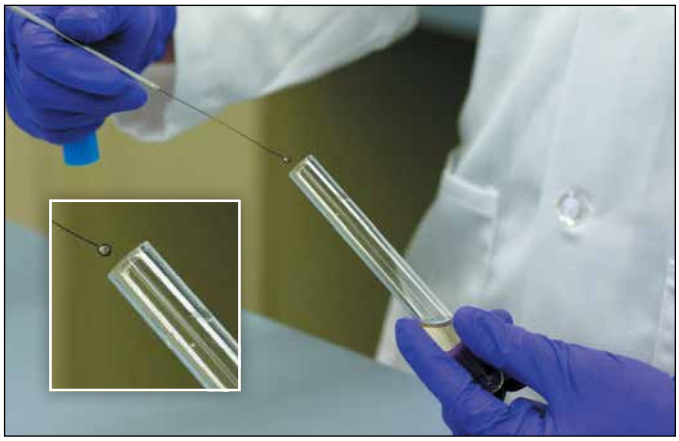
1.18 Vortex Mixer ■ Bacteria may be suspended in a broth using a vortex mixer. The switch on the bottom has three positions: "auto" (left), "off" (center), and "on" (right). The rubber boot is activated when touched only if the "auto" position is used; "on" means the boot is constantly vibrating. Above the on/off/auto switch is a variable speed knob. The slowest speed that allows the vortex to reach the bottom of the tube is used. Caution must be used to prevent broth from getting into the cap or losing control of the tube and causing a spill (note the hand position around the tube, ready to grab it). Short bursts of vortexing can be used if the glassware is too full to allow vortexing to the bottom.

1.20 Removing the Tube Cap ■ The loop is held in the dominant hand and the tube in the other hand. Remove the tube's cap with the little finger of your loop hand by pulling the tube away with the other hand; keep your loop hand still. Hold the cap in your little finger during the transfer. When replacing the cap, move the tube back to the cap to keep your loop hand still. The replaced cap does not have to be on firmly at this time—just enough to cover the tube.

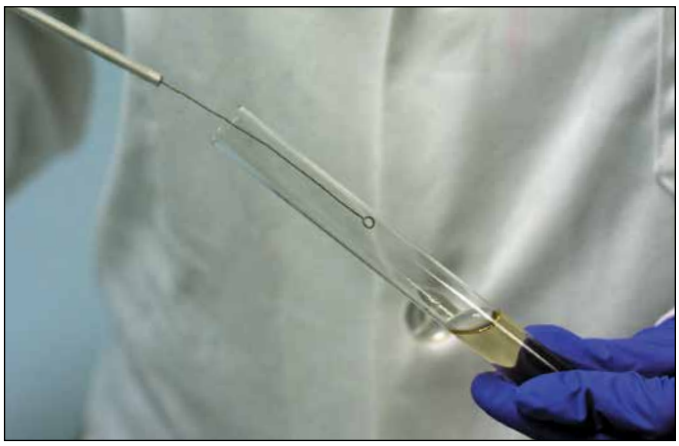
1.22 Holding the Tube at an Angle ■ The tube is held at an angle to minimize the chance that airborne microbes will drop into it. Notice that the tube's cap is held in the loop hand.



1.23 Move the Tube, Not the Loop ■ The open tube is held at an angle to minimize airborne contamination of it. When placing a loop into a broth tube or removing it, keep the loop hand still and move the tube. ***Be careful not to catch the loop on the tube's lip when removing it***. This produces aerosols that can be dangerous or produce contamination.



1.24 Removing the Loop from Broth ■ Notice the film of broth in the loop (see inset). Be careful not to catch the loop on the lip of the tube when removing it. This would produce aerosols and droplets that can be dangerous or produce contamination.

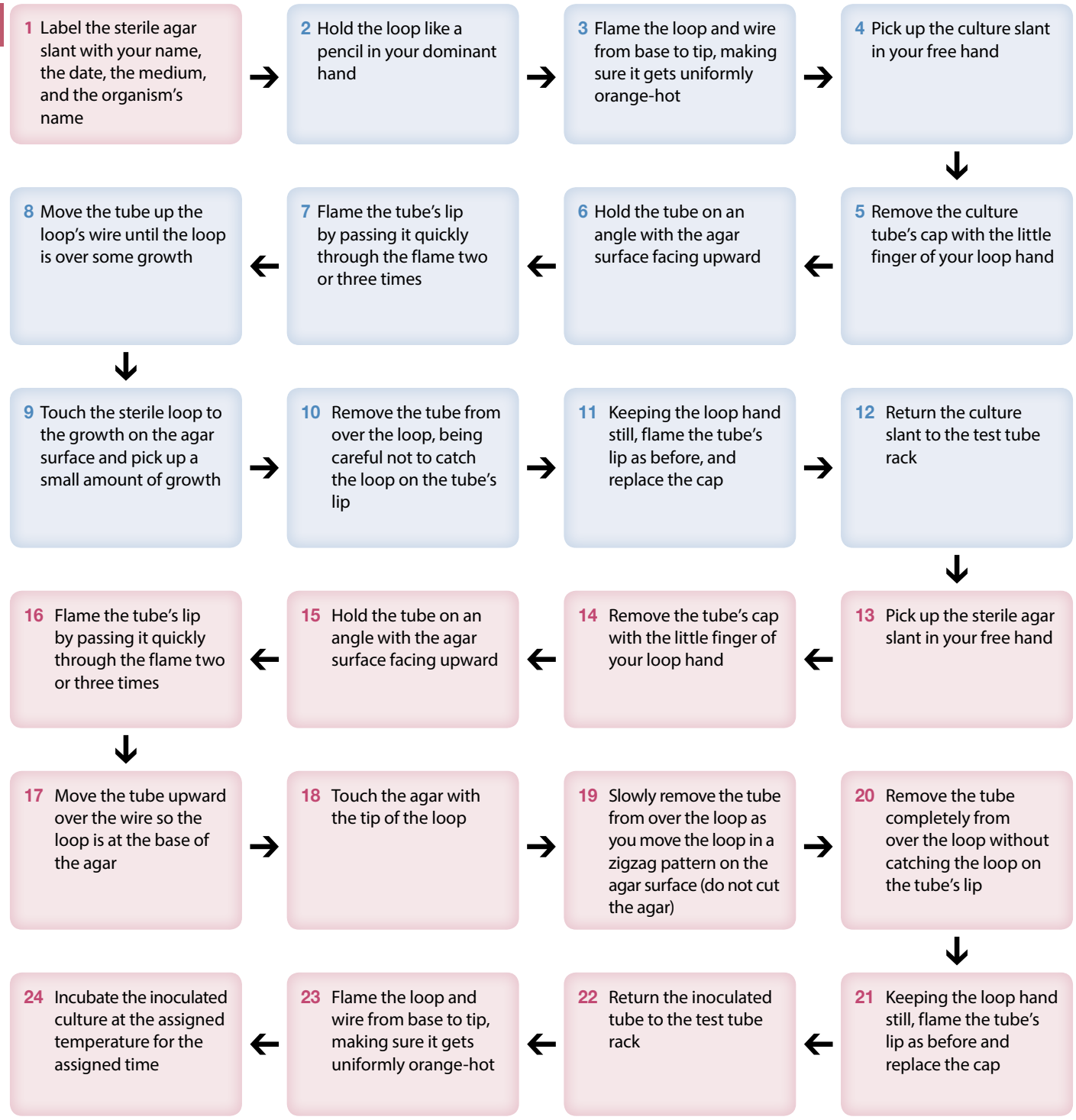


1.25 Removing Excess Broth from Loop ■ Before removing it from the new culture tube, tap the *face* of the loop on the glass to remove the broth film (Fig. 1.24). Failing to do so will result in splattering and aerosols when sterilizing the loop in a flame.

Transfer from an Agar Slant Culture to a Sterile Agar Slant

As you read these instructions, also follow the procedural diagram in Figure 1.26 to get a summary view of the process. This transfer has a lot in common with the broth-to-broth transfer and you are referred back to it at relevant points. **Make appropriate adjustments if handling a BSL-2 organism.**

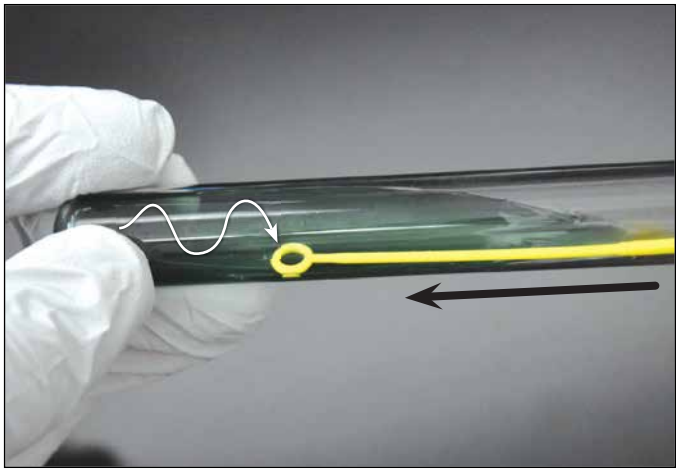
1. Label the sterile agar slant with your name, the date, the medium, and the inoculum.
2. Hold the loop like a pencil and then flame it from base to tip as in a broth transfer (Fig. 1.17). Be sure the entire wire becomes orange-hot at some point.
3. Loosen and remove the cap on the culture tube as in a broth transfer (Fig. 1.20).
4. Hold the culture tube on an angle with the agar surface facing upward.
5. Flame the tube's lip as in a broth transfer (Fig. 1.21).
6. Move the culture tube up the wire of the loop, and then gently touch the loop's tip to the growth on the agar's surface (Fig. 1.27). You don't need to dig into the agar, nor do you need to scoop up a glob of growth. Just touch the loop to the growth and pick up the smallest amount you can see with your naked eye.
7. Holding your loop hand still, carefully remove the tube from over the wire, flame the culture tube's lip, and replace its cap. Place the culture in the test tube rack.
8. Pick up the sterile agar slant in your free hand, remove the cap, and flame the tube as before.
9. Holding the tube on an angle with the agar surface upward, move the tube over the wire so the loop is near the bottom of the slant.
10. Touch the tip of the loop (where there is organism) to the agar. Then, as you withdraw the tube move the loop back and forth (Fig. 1.28). Be careful not to cut the agar with the loop. This is called a **fishtail inoculation** or **fishtail streak** because you are seeding the agar surface in a wavy pattern resembling the movement of a fish tail.
11. Be careful not to catch the loop on the tube's lip as you remove the tube. Then, keeping the loop hand still, flame the tube's lip, replace its cap, and put it in the test tube rack.
12. Flame the loop from base to tip as before.
13. Incubate the inoculated culture at the assigned temperature for the assigned time.



- Actions involving the agar slant pure culture are in **BLUE**
- Actions involving the sterile agar slant are in **RED**

1.26 Procedural Diagram: Aseptic Transfer from a Nutrient Agar Slant to a Sterile Nutrient Agar Slant ■ This is a summary of the procedure. Make every effort to keep your loop hand as still as possible throughout the transfer. Details can be found in the text. Make appropriate adjustments if transferring a BSL-2 organism.

1.27 A Loop and an Agar Slant ■ When placing a loop into a slant tube or removing it, the loop hand is kept still while the tube is moved. Hold the tube so the agar is facing upward. To pick up the inoculum, you only need to gently touch the growth on the agar surface.

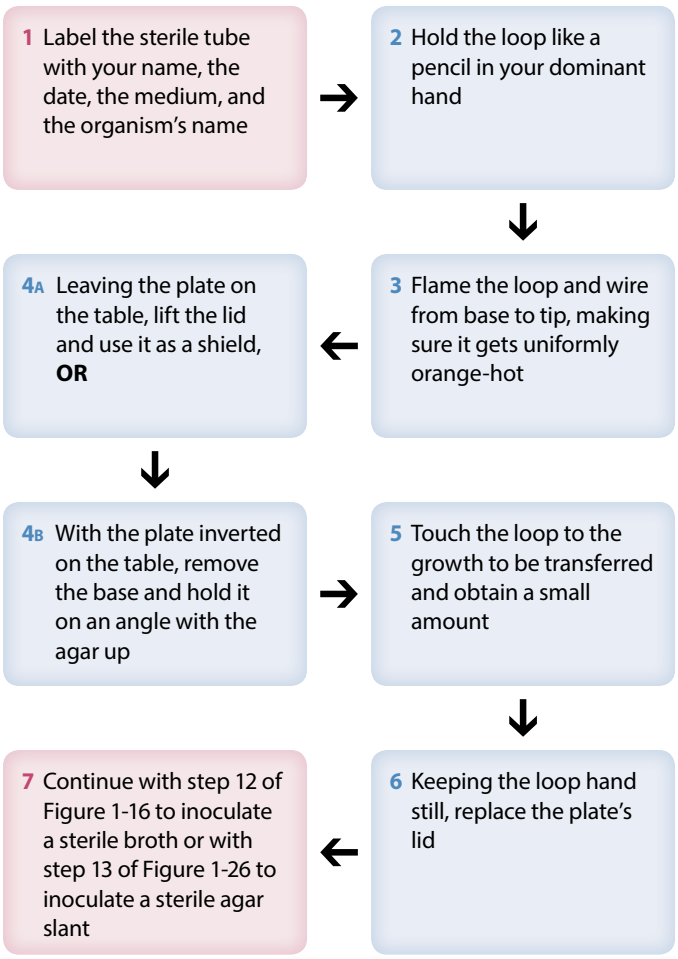


1.28 Fishtail Inoculation of a Slant ■ Begin at the base of the slant and gently move the loop back and forth as you withdraw the tube. Use the end of the loop and be careful not to cut the agar. After completing the transfer, sterilize the loop or dispose of it properly. (A disposable loop was used in this photo.)

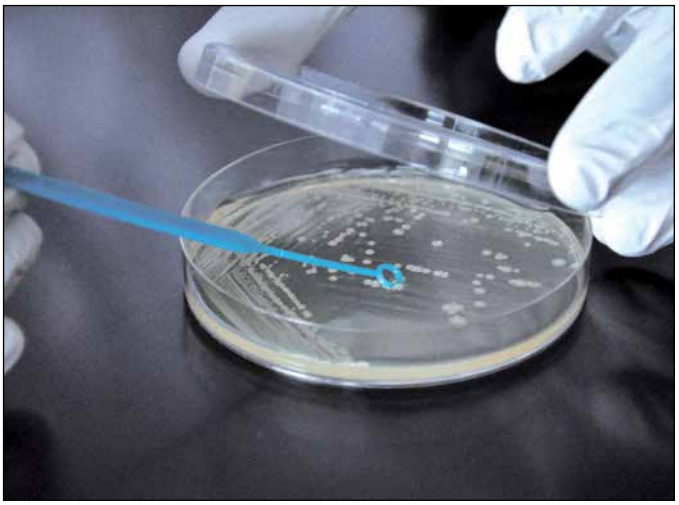
Transfers from a Plate Culture to a Sterile Broth or Agar Slant

As you read these instructions, also follow the procedural diagram in Figure 1.29 to get a summary view of the process. **Make appropriate adjustments if transferring a BSL-2 organism.**

1. Label the sterile broth tube with your name, the date, the medium, and the organism you are inoculating it with.
2. Flame the loop from base to tip (Fig. 1.17).
3. Lift the lid of the Petri dish and use it as a shield from airborne contamination (Fig. 1.30).
4. Touch the loop to an uninoculated portion of the plate to cool it. (Loop wires can get very hot if a series of transfers are made in a short period of time. Placing a hot wire on growth may cause the growth to spatter and create aerosols. If you are not doing a lot of successive transfers, this is probably unnecessary.) Gently touch the loop to the center of an isolated colony on the agar surface and collect the smallest amount you can see. As with the slant, you don't need to dig into the agar or scoop up a glob of growth.
5. Remove the loop and replace the lid.
6. Pick up the sterile broth and continue with step 9 in the broth-to-broth transfer (p. 34) or step 12 in Figure 1.16. If transferring to a slant, continue with step 8 in the slant-to-slant transfer (p. 37) and step 13 in Figure 1.26.



1.29 Procedural Diagram: Aseptic Transfer from a Nutrient Agar Plate Pure Culture to a Sterile Tubed Medium ■ This is a summary of the procedure. Make every effort to keep your loop hand as still as possible throughout the transfer. Details can be found in the text. Inoculation of a sterile broth is the same as in Figure 1.16, whereas inoculation of a sterile slant is the same as in Figure 1.26. Make appropriate adjustments if transferring a BSL-2 organism.



1.30 "Picking" a Colony for Transfer ■ Touch the tip of the loop to the center of an isolated colony and get a small amount of growth. Use the lid as a shield from airborne contamination.

■ Application

To be a successful microbiologist, you must be able to transfer microorganisms from one place to another aseptically.

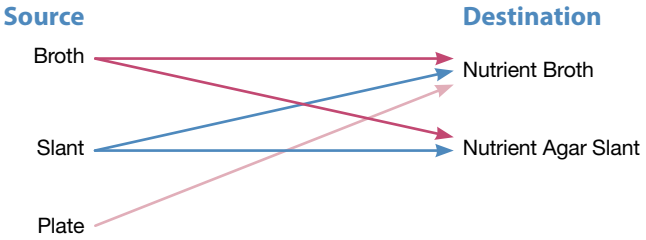
■ In This Exercise

You now have the procedures for obtaining growth from a broth culture, an agar slant culture, and a plated culture. You also have the procedures for inoculating a sterile broth and a sterile agar slant. These can be performed in any combination necessary. Today, you will begin by testing your baseline dexterity by using sterile agar and sterile broth as the "culture" tubes *and* the tubes to be inoculated. After a little practice with these "blanks," you will do transfers with real cultures and sterile media in the following combinations: agar slant culture to sterile agar slant and sterile broth, broth culture to sterile agar slant and sterile broth, and plate culture to sterile broth (Fig. 1.31).

▼ Materials

Per Student

- □ Lab coat
- □ Disposable gloves
- □ Chemical eye protection
- □ Inoculating loop
- □ Bunsen burner
- □ Four sterile nutrient broth tubes
- □ Three sterile nutrient agar slants
- □ Marking pens and labeling tape (or materials for the preferred labeling method in your laboratory)
- □ (Optional) vortex mixer



1.31 Procedural Diagram for this Lab ■ In today's lab, you will make transfers from broth and slant cultures of *S. epidermidis* to sterile nutrient broth and nutrient agar slant media. You will also transfer from a plate culture of *S. epidermidis* to a nutrient broth.

Per Student Group

- □ Nutrient agar (NA) slant culture of *Staphylococcus epidermidis*
- □ Nutrient broth (NB) culture of *Staphylococcus epidermidis*
- □ Nutrient agar streak plate of *Staphylococcus epidermidis*

PROCEDURE

Lab One

1. Wear a lab coat, gloves, and chemical eye protection when performing this procedure.
2. Label one nutrient broth (NB) and one nutrient agar (NA) tube with your name and the word "sterile."
3. Using the sterile NB tube and the sterile NA tube, practice making transfers between them in all possible combinations. Your lab partner has the same assignment using a second set of tubes. Work with her/him and alternate transferring and evaluating each other's technique. (Not only will you learn by doing and evaluating another person, alternating will allow the tubes to cool.)
4. Incubate the "sterile" practice tubes at 35 ± 2°C until the next lab period. (You may wait to do this until you have completed step 5 and then transfer all the tubes to the incubator at one time.)
5. Once you have been "cleared" by your instructor to make transfers using real cultures, each student should perform the following, using Figure 1.31 as a guide:
   a. Label a sterile nutrient agar slant and a sterile nutrient broth with your name, the medium in the tube (either NA or NB), the source of inoculum (NA slant), and the organism. Then, aseptically transfer from the *S. epidermidis* NA slant culture to the sterile NA slant and the sterile NB.