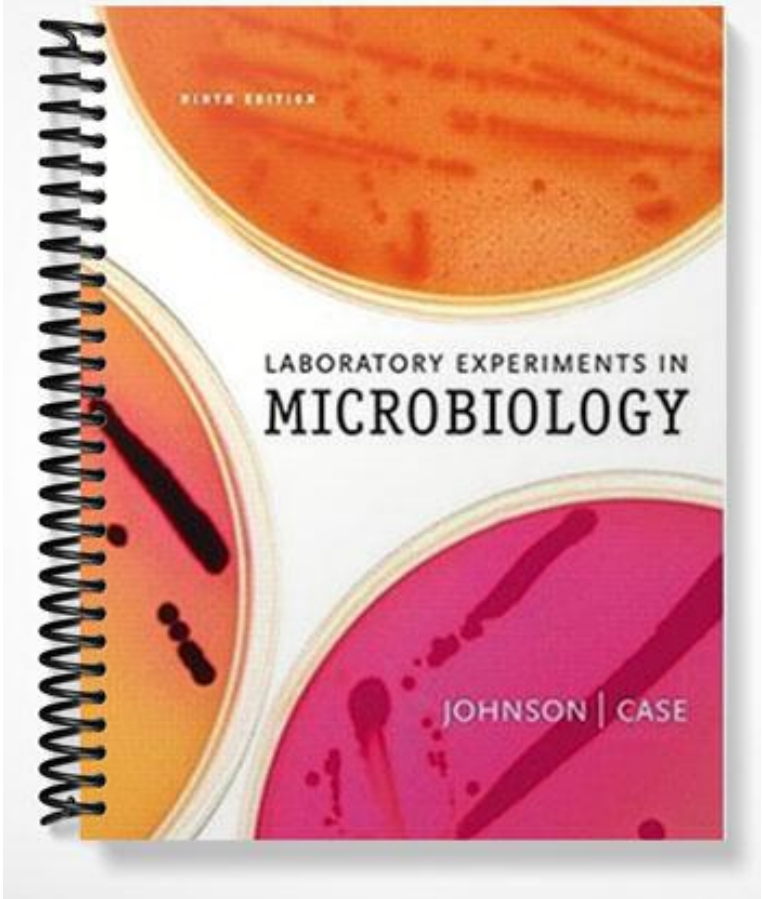
SOLUTIONS MANUAL



PREPARATION GUIDE FOR

LABORATORY EXPERIMENTS IN MICROBIOLOGY

NINTH EDITION

CHRISTINE L. CASE

Skyline College

TED R. JOHNSON

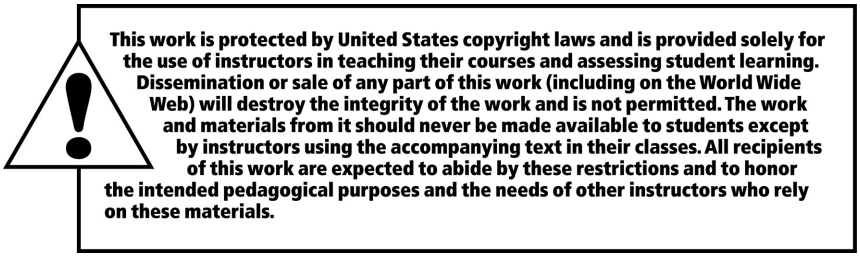
St. Olaf College

Benjamin Cummings

San Francisco Boston New York
Cape Town Hong Kong London Madrid Mexico City
Montreal Munich Paris Singapore Sydney Tokyo Toronto

Executive Editor: Leslie Berriman
Development Manager: Barbara Yien
Assistant Editors: Katie Heimsoth and Kelly Reed
Managing Editor: Wendy Earl
Production Editor: Leslie Austin
Copyeditor: Sally Peyrefitte
Compositor: The Left Coast Group
Cover Design: Riezebos Holzbaur Design Group
Proofreader: Martha Ghent
Image Rights and Permissions Manager: Zina Arabia
Senior Manufacturing Buyer: Stacey Weinberger
Senior Marketing Manager: Neena Bali

Cover Photo Credit: Andrew Brookes/CORBIS



Benjamin Cummings
is an imprint of

PEARSON

www.pearsonhighered.com

ISBN 10: **0-321-57566-0**; ISBN 13: **978-0-321-57566-1**

1 2 3 4 5 6 7 8 9 10—**B&B**—13 12 11 10 09
Manufactured in the United States of America.

Contents

To the Instructor

The exercises in *Laboratory Experiments in Microbiology,* Ninth Edition, are designed to reinforce lecture material and to provide an opportunity for students to (1) master microbiological techniques, (2) develop critical thinking skills, and (3) learn to analyze and present data. For example, the exercises in Part Five, Microbial Metabolism, build logically to the traditional identification of an unknown (Exercises 18, 50, and 51). Moreover, these exercises are essential for understanding the metabolic processes covered in lecture.

Most of the exercises in *Laboratory Experiments in Microbiology* are designed to take 60 to 75 minutes. Groups of exercises may be assigned for longer laboratory periods, and parts of exercises may be assigned to suit your class objectives. Most exercises require all or a portion of two laboratory periods of about 75 minutes each. Media are inoculated during the first period, and results are recorded during the second period. Some students can complete their reports during laboratory time, but most prefer to work on their reports outside the laboratory. We usually require that completed laboratory reports be turned in at the end of the period following completion of the exercise.

Exercises are designed to conserve media whenever possible. This Preparation Guide includes a Materials list for each exercise. Materials are divided into "Per Two Students," "Per Laboratory," and "Per _____ Students." Requirements for the latter will depend on the assignment.

All the exercises have been class tested. Microscopy (Exercises 1 and 2) and unknown identification (Exercises 10, 18, 50, and 51) are usually performed by the students individually. We recommend that students work in pairs for the other exercises, except where larger teams are specified. Some instructors have found groups of three students to be optimal in some instances.

Core Curriculum

The American Society for Microbiology (ASM)* endorses the following laboratory core curriculum, considered essential to teach in every introductory microbiology laboratory regardless of its emphasis. The core curriculum includes laboratory skills, laboratory thinking skills, and safety.

Each element of ASM's Laboratory Core Curriculum is covered in several exercises in *Laboratory Experiments in Microbiology.* The following laboratory skills recommended by ASM are used throughout *Laboratory Experiments in Microbiology.*

A student successfully completing basic microbiology will demonstrate ability to:	Introduced in *Laboratory Experiments in Microbiology* in Exercise No.
1. Use a *brightfield light microscope* to view and interpret slides, including	1
a. correctly setting up and focusing the microscope	
b. properly handling, cleaning, and storing the microscope	
c. correctly using all lenses	
d. recording microscopic observations	

* ASM Curriculum Recommendations for Introductory Microbiology. Available at www.asm.org

	Exercise No. (continued)
2. *Properly prepare slides* for microbiological examination, including	
a. cleaning and disposing of slides	5
b. preparing smears from solid and liquid cultures	5
c. performing wet-mount and/or hanging-drop preparations	2
d. performing Gram stains	7
3. *Properly use aseptic techniques* for the transfer and handling of microorganisms and instruments, including	
a. sterilizing and maintaining sterility of transfer instruments	4
b. performing aseptic transfer	4
c. obtaining microbial samples	11
4. *Use appropriate microbiological* media and test systems, including	
a. isolating colonies and/or plaques	11
b. maintaining pure cultures	11
c. using biochemical test media	12
d. accurately recording macroscopic observations	3
5. *Estimate the number of microbes* in a sample using serial dilution techniques, including	11, 37, 41, 54
a. correctly choosing and using pipettes and pipetting devices	
b. correctly spreading diluted samples for counting	
c. estimating appropriate dilutions	
d. extrapolating plate counts to obtain the correct number of colony-forming units (cfu) or plaque-forming units (pfu) in the starting sample	
6. *Use standard microbiology* laboratory equipment correctly, including	
a. using the standard metric system for weights, lengths, diameters, and volumes	3, 11
b. lighting and adjusting a laboratory burner	4
c. using an incubator	3

Laboratory Safety

Adherence to safety procedures not only minimizes risk of infections in the laboratory, but also trains students to minimize the transmission of nosocomial infections in their future work environments. The ASM laboratory core curriculum recommends that a student successfully completing basic microbiology demonstrate the ability to explain and practice safe (1) microbiological procedures, (2) protective procedures, and (3) emergency procedures.

Laboratory Safety Skills are listed on pp. x–xiii of *Laboratory Experiments in Microbiology* and are integrated into every lab exercise to provide students with repetition and an opportunity to master laboratory safety.

Most laboratory-acquired infections are probably transmitted by inhaled aerosols. We have avoided use of respiratory pathogens; in fact, most of the microbes chosen for the exercises are nonpathogens.

However, many normally innocuous microbes can be opportunistic pathogens, and employing safety procedures is essential. The following guidelines, applicable to teaching facilities, are adapted from the Centers for Disease Control and Prevention (CDC) guidelines.* The CDC also recommends preparing or adopting a biosafety manual.

* U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories*. Available from www.cdc.gov

We recommend that the instructor develop a list of safety procedures applicable to his or her lab and distribute it to each student. In this way, the issue of safety will be brought to the individual student's attention.

Lab Coats

We require lab coats in our labs. Alternatives to expensive coats are the following:

1. Nonflammable paper coats designed for microbiology lab use, manufactured by Kimberly-Clark.
2. Patient gowns, for students working in hospitals.
3. Lab coats from former students, stored and supplied by the stockroom, rented or sold to new students.
4. Oversized white shirts.

Safety Glasses

Students are required to have safety glasses for chemistry and can use these in microbiology labs where hazardous chemicals, ultraviolet radiation, or blood are used (Exercises 23, 28, 30, 31, 32, and 42).

Gloves

Gloving for general microbiologic work should be discouraged. Students will bring one pair of gloves and reuse them. Reuse increases the risk of contact with any microbes on the gloves.

Students should wear gloves for staining with ethidium bromide (Exercises 30 and 31) and handling blood other than the student's own blood (Exercise 42).

Standard Microbiological Practices

1. Work surfaces are decontaminated at the beginning and end of every lab period and after any spill of viable material.
2. Mechanical pipetting devices are used; mouth pipetting is prohibited.
3. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.
4. Students wash their hands after handling microbiological cultures and every time they leave the laboratory.
5. All contaminated material is decontaminated, preferably by autoclaving, before being discarded.
6. Laboratory clothes that protect street clothing (e.g., solid-front or wraparound gowns) are worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated (e.g., by autoclaving) before it is laundered.

Disinfection

For routine handwashing when not working with infectious agents, students can use any liquid or powdered soaps. Bar soaps should not be used. Liquid soaps that lack preservatives should be cleaned out routinely and replaced with new soap. Powdered soaps offer two advantages; they do not become contaminated, nor do they allow organisms to grow in them.

Rapid disinfection of hands may be accomplished by any of the following:

- Wescodyne-detergent preparation; scrub for 30 to 60 seconds, and then rinse with water.
- 4% chlorhexidine-detergent; scrub for 30 to 60 seconds, and then rinse with water.
- A phenolic disinfectant-detergent; scrub for 20 to 30 seconds, and then rinse with water.
- Alcohol (50% to 70%); apply for 20 to 30 seconds, followed by a soap scrub of 10 to 15 seconds, and then rinse with water.

Benchtops may be disinfected by using a disinfectant-detergent according to the manufacturer's directions. If 0.5% hypochlorite (household bleach) is used, remember that it is easily neutralized by organic matter, may discolor surfaces, is corrosive to metals, and has a disagreeable odor.

Special Practices

1. Laboratory doors are kept closed when experiments are in progress.
2. The instructor controls access to the laboratory and limits access only to persons whose presence is required for program or support purposes.
3. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable, leakproof container. The container is closed before it is removed from the laboratory.
4. An insect and rodent control program is in effect.
5. A needle should not be bent, replaced in the sheath, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant "Sharps" container and decontaminated, preferably by autoclaving, before being discarded or reused.
6. Students who become pregnant, are taking immunosuppressive drugs, or have any other medical condition (e.g., diabetes, immune system disorder) that might necessitate special precautions in the laboratory must inform the instructor.

Laboratory Facilities

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

About Aerosols

Instruct students to minimize production of and contact with aerosols. Aerosols can be generated by the following procedures:

Procedure	Aerosol Generated By
Blood handling	Forcing blood through needle and taking needle off syringe; squirting blood into container; vibrating needle.
Mixing or shaking	Inevitable production of stable aerosols. Splashing.
Transport	Aerosol produced in airspace in tube by shaking is released when the tube is opened.
Opening containers	An ever-present and inevitable danger.
Centrifugation	Tubes leaking or breaking; aerosol in airspace released when the tube is opened.
Pipetting	Dispensing last drop; dropping culture onto bench or plates.
Bacteriologic loops	Hot loops sizzle; vibrating loop; plate streaking; flaming loop.
Disposal and washup	Almost all activities.

Contact with Blood and Other Body Fluids

The following procedures* should be used by all health care workers, including students, whose activities involve contact with patients or with blood or other body fluids. Although these procedures were developed to minimize the risk of transmitting human immunodeficiency virus (HIV) and serum hepatitis in a health care environment, adherence to these guidelines will minimize transmission of *all* nosocomial infections.

1. Students should work only with their own blood, saliva, feces, and urine.
2. Gloves should be worn for touching others' blood or body fluids, mucous membranes, or nonintact skin and for handling items or surfaces soiled with blood or body fluids.
3. Hands and other skin surfaces should be washed immediately and thoroughly if they are contaminated with blood or other body fluids.
4. Masks and protective eyewear or face shields should be worn during procedures that are likely to generate droplets of blood or other body fluids.
5. Gowns or aprons should be worn during procedures that are likely to generate splashes of blood or other body fluids.
6. Hands and other skin surfaces should be washed immediately after gloves are removed.
7. To prevent needle-stick injuries, needles should not be recapped, purposely bent or broken, or otherwise manipulated by hand. After they are used, disposable syringes and needles, scalpel blades, and other sharp items should be placed in puncture-resistant containers for disposal.
8. Pregnant students are not known to be at greater risk of contracting HIV infection than students who are not pregnant; however, if a student develops HIV infection during pregnancy, the infant is at risk of infection. Because of this risk, pregnant students should be especially familiar with and strictly adhere to precautions to minimize the risk of HIV transmission.

The First Laboratory Period

During the first period, we suggest that you assign laboratory lockers and cover the material in the lab manual's Introduction. Make the following materials available to the students:

Bergey's Manual of Determinative Bacteriology and/or *Bergey's Manual of Systematic Bacteriology*
Burner or electric incinerator
Compound light microscope (with oil immersion lens)
Coverslips
Disinfectant in wash bottle
Dissecting microscope
Distilled water in wash bottle
Glass microscope slides
Gram-staining reagents
Immersion oil
Incubator, 35°C
Inoculating loop and needle
Isopropanol alcohol and small beakers
Lens paper
Methanol (95%) for fixing bacterial smears
Propipette or pipette bulb
Rubber bands
Staining dish or rack
Stapler

* Centers for Disease Control and Prevention. "Recommendations for Prevention of HIV Transmission in Health-Care Settings." *MMWR* 36(S2), 1987.

Test-tube rack or cans
To Be Autoclaved area with acetone for removing markings
Tripod and asbestos pad or hot plate
Wax marking pencils
Wooden clothespins

These materials are required throughout the course. They are listed in Materials only the first few times they are used.

Autoclaving

Sterile materials and supplies are required for most of the exercises. Cotton swabs can be autoclaved in test tubes or in wrapping material. Odd-sized supplies, such as beakers and membrane filter apparatuses, will require special wrapping.

A list of acceptable wrapping materials for use in an autoclave follows:

Autoclavable cellophane
Glassine envelopes
Kraft paper (30–40 weight)
Muslin (30 weight)
Paper bags, small

These materials have sufficiently high permeability ratings to allow transfer of moisture. Aluminum foil is not permeable to steam. Polyethylene is permeable to steam but not listed because it is difficult to remove air from packages.

Dilution Techniques and Calculations

The problems in Appendix B, Dilution Techniques and Calculations, can be assigned before an exercise requiring dilutions.

The Dissecting Microscope

The dissecting microscope is suggested to students as an aid in observing colonies and multicellular organisms. The microscope is described in Appendix E, Use of the Dissecting Microscope, and a few questions have been included to help students become familiar with this microscope.

Disposal Area

The following items should be in the lab and easily accessed by students:

1. Several clearly labeled biohazard containers containing red biohazard bags. Biohazard bags are autoclaved before being discarded.
2. "Sharps" containers for syringes, needles, scalpels, and other sharp material. Commercial medical waste companies can be utilized to dispose of the autoclaved full "Sharps" containers.
3. Containers for noncontaminated broken glass should be available in the lab.
4. Pipettes can be placed in containers of disinfectant.
5. A "chemical disposal" jar for used stains should be available.

Field Trips

Occasional field trips are invaluable because they provide students with an opportunity to acquire practical information as well as a chance to look at prospective careers in microbiology. Although they are not reflected in the suggested laboratory schedules, we recommend taking at least one field trip during the semester. Following are suggestions:

Sewage treatment plant
Water treatment plant
Mushroom farm
Winery or brewery (with a visit to the laboratory)
Microbiology laboratory of the state public health department
Cheese-making or milk-processing plant
Clinical microbiology laboratory
Microbiology laboratory of an agricultural research division of a state or federal agriculture department, or of the U.S. Food and Drug Administration

Graphing

The activity in Appendix D, Graphing, can be used instead of, or with, the laboratory growth experiment (Exercise 20) to introduce growth curves.

Incubators

The following temperatures are required for various exercises:

15°C	Exercise 20, Determination of a Bacterial Growth Curve: The Role of Temperature. Many refrigerators can be raised to this temperature.
20°C to 28°C	Room temperature can be used for this range if incubators are not available.
35°C	The optimum growth temperature of most mesophiles commonly cultivated in the laboratory, and the standard incubation temperature used in the manual.
44.5°C	Exercises 52 and 53 for counting fecal coliforms.
45°C	Exercise 20, Determination of a Bacterial Growth Curve: The Role of Temperature, and Exercise 55, Microbes Used in the Production of Foods.
55°C	Exercise 20, Determination of a Bacterial Growth Curve: The Role of Temperature.

Inoculating Loops

Use of the inoculating loop is introduced in Part Two of the lab manual; loops and needles are described and used in Exercise 4, Transfer of Bacteria: Aseptic Technique.

Membrane Filter

Students are referred to Appendix F, Use of the Membrane Filter, for instructions on use of membrane filtration equipment. Instructions are given for Millipore membrane filters. Membrane filtration equipment is manufactured by the following companies:

Pall Corporation www.pall.com	See materials in *Microbial Analysis of Water*.
Millipore Corporation www.millipore.com	See *Membrane Filtration*.

Nalge Nunc International www.nalgenunc.com	Presterilized, disposable Nalgene Analytical Filter Unit and Filter Funnel.

Pipetting

Appendix A includes instructions on pipetting. Students are told to use a propipette or pipette bulb of some type. No one should ever pipette by mouth.

Disposable micropipette tips can be calibrated (and labeled with a marker) with a micropipette such as Pipetman. Students can use the calibrated tips with one-piece plastic Pasteur pipettes.

pH Indicators Used in Microbiology

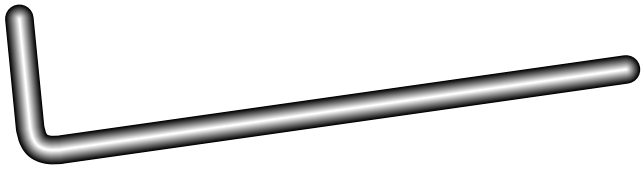
		Color	
Indicator	**pH Range**	**Acidic**	**Alkaline**
Acid fuchsin	5.0 to 8.0	Pink	Pale Yellow
Brilliant green	0.0 to 2.6	0.0 Yellow 2.6 Green	Green
Bromcresol green	3.8 to 5.4	Yellow	Blue-green
Bromcresol purple	5.2 to 6.8	Yellow	Purple
Bromthymol blue	6.0 to 7.6	Yellow	Blue
Congo red	3.0 to 5.0	Blue-violet	Red
Cresol red	2–3 to 8.8	Orange	7.2 Yellow 8.8 Red
Litmus	4.5 to 8.3	Red	Blue
Methyl red	4.4 to 6.2	Red	Yellow
Phenol red	6.8 to 8.4	Yellow	Red

Solid Culture Media in Petri Plates

When a solid culture medium in a Petri plate is required, this Preparation Guide lists it as nutrient agar plate, eosin methylene blue (EMB) plate, etc. If you do not wish to supply poured plates, provide tubes or bottles of culture media, and the students can pour their own plates. To save class time, store media in a 50°C water bath for the students.

Spreading Rods

Spreading rods can be made by bending a glass rod or Pasteur pipette (see the following illustration and Figure 27.4 in the lab manual). A spreader can also be made by placing a piece of No. 16 shrinkable Teflon tubing over a large, straightened paper clip (see *Manual of Methods for General and Molecular Bacteriology*, American Society for Microbiology). Metal spreaders can be purchased from Carolina Biological Supply or Arben Bioscience (www.arbenbio.com).



Spreading rods are used in Exercises 27, 28, 29, 31, and 57. They may be disinfected by dipping them in a small beaker of isopropyl alcohol and burning off the alcohol.

Staining

We use plastic staining trays to support slides and collect wasted stain. Students discard the stain from the trays into a chemical disposal container. Stainless steel and plastic staining trays can be purchased from biologic suppliers.

A handmade staining rack can be made by using two pipettes (1 ml to 5 ml) or glass tubes held together with a short piece of rubber tubing at each end.

Suppliers

We have provided the names and addresses of suppliers whenever appropriate. Most supplies can be obtained from the following sources:

BBL and Difco
BD Bioscience
www.bdbiosciences.com

Carolina Biological Supply
www.carolina.com

Fisher Scientific
www.fishersci.com

Hardy Diagnostics
www.hardydiagnostics.com

ProLab Diagnostics
www.pro-lab.com

Sigma Chemical Co.
www.sigmaaldrich.com

Ward's Natural Science Establishment
http://wardsci.com

Techniques and Time Requirements for Each Exercise

Exercise Number and Title	Level	Techniques Introduced	Techniques Required	Time Required, in Hours		
				1st Lab Period	2nd Lab Period	3rd Lab Period
1 Use and Care of the Microscope	Basic	Brightfield Microscopy	None	1.5		
2 Examination of Living Microorganisms	Basic	Hanging drop, wet mount Phase-contrast microscopy	Compound light microscopy, Ex. 1	1.5		
3 Microbes in the Environment	Basic	Culture media	Pipetting, App. A	0.75	0.75	
4 Transfer of Bacteria: Aseptic Technique	Basic	Culture media preparation, aseptic transfer techniques, inoculating loop and needle	Compound light microscopy, Ex. 1 Wet mount, Ex. 2	1.0	0.75	
5 Preparation of Smears and Simple Staining	Basic	Smear preparation, inoculating loop	Compound light microscopy, Ex. 1 Inoculating loop, Ex. 4	1.5		
6 Negative Staining	Basic	Negative stain	Compound light microscopy, Ex. 1 Smear preparation, Ex. 5	1.0		
7 Gram Staining	Basic	Gram stain	Compound light microscopy, Ex. 1 Smear preparation, Ex. 5 Simple Staining, Ex. 5	1.0		
8 Acid-Fast Staining	Enrichment	Acid-fast stain	Compound light microscopy, Ex. 1 Smear preparation, Ex. 5 Simple Staining, Ex. 5	1.0		

Exercise Number and Title	Level	Techniques Introduced	Techniques Required	Time Required, in Hours		
				1st Lab Period	2nd Lab Period	3rd Lab Period
9 Structural Stains (Endospore, Capsule, and Flagella)	Enrichment	Endospore, capsule, flagella stain	Compound light microscopy, Ex. 1 Smear preparation, Ex. 5 Simple Staining, Ex. 5 Negative staining, Ex. 6	1.0		
10 Morphologic Unknown	Enrichment	Introduction to bacterial indentification	Compound light microscopy, Ex. 1 Hanging-drop and wet-mount procedures, Ex. 2 Smear preparation, Ex. 5 Simple staining, Ex. 5 Negative staining, Ex. 6 Gram staining, Ex. 7 Acid-fast staining, Ex. 8 Endospore, capsule, and flagella staining, Ex. 9	1.0	1.0	
11 Isolation of Bacteria by Dilution Techniques	Basic	Streak plate, pour plate	Compound light microscopy, Ex. 1 Aseptic technique, Ex. 4 Pipetting, App. A Serial dilution techniques, App. B	1.5	0.5	0.5
12 Special Media for Isolating Bacteria	Basic	Selective media	Compound light microscopy, Ex. 1 Aseptic technique, Ex. 4 Inoculating loop, Ex. 4 Smear preparation, Ex. 5 Gram staining, Ex. 6 Plate streaking, Ex. 11	0.5	0.5	
13 Carbohydrate Catabolism	Basic	Starch hydrolysis, OF-glucose	Inoculating loop and needle, Ex. 4 Aseptic technique, Ex. 4	0.5	1.0	
14 Fermentation	Basic	Fermentation test, MRVP test, citrate test	Inoculating loop, Ex. 4 Aseptic technique, Ex. 4 OF test, Ex. 13	0.5	0.5	
15 Protein Catabolism, Part 1	Basic	Gelatin hydrolysis, urea hydrolysis	Inoculating loop, Ex. 4 Aseptic technique, Ex. 4	0.5	0.5	0.25
16 Protein Catabolism, Part 2	Basic	Phenylalanine deamination, hydrogen sulfide, MIO	Inoculating loop, Ex. 4 Aseptic technique, Ex. 4	0.5	0.5	
17 Respiration	Basic	Nitrate reduction test, oxidase test, catalase test	Inoculating loop, Ex. 4 Aseptic technique, Ex. 4 Plate streaking, Ex. 11 Carbohydrate catabolism, Ex. 13	0.5	0.5	

Exercise Number and Title	Level	Techniques Introduced	Techniques Required	Time Required, in Hours		
				1st Lab Period	2nd Lab Period	3rd Lab Period
18 Unknown Identification and *Bergey's Manual*	Enrichment	Identification keys for bacteria	Compound light microscopy, Ex. 1 Hanging drop, Ex. 2 Wet mount, Ex. 2 Inoculating loop and needle, Ex. 4 Aseptic technique, Ex. 4 Negative staining, Ex. 6 Gram staining, Ex. 7 Acid-fast staining, Ex. 8 Endospore, capsule, and flagella staining, Ex. 9 Plate streaking, Ex. 11 OF test, Ex. 13 Starch hydrolysis, Ex. 13 MRVP tests, Ex. 14 Fermentation tests, Ex. 14 Protein catabolism, parts 1 and 2, Ex. 15 and 16 Catalase test, Ex. 17 Nitrate reduction test, Ex. 17 Oxidase test, Ex. 17	0.5	1.0	1.5
19 Oxygen and the Growth of Bacteria	Basic	Anaerobic jar, thioglycolate	Inoculating loop, Ex. 4 Aseptic technique, Ex. 4 Plate streaking, Ex. 11 Selective media, Ex. 12 Catalase test, Ex. 17	0.5	0.5	0.5
20 Determination of a Bacterial Growth Curve: The Role of Temperature	Basic	Spectrophotometry, graphing	Aseptic technique, Ex. 4 Pipetting, App. A Spectrophotometry, App. C Graphing, App. D	1.5		
21 Biofilms	Enrichment		Compound Light Microscopy Ex. 1 Smear fixing, Ex. 5 Simple Staining, Ex. 5	0.5	0.25	1.5
22 Physical Methods of Control: Heat	Basic	Graphing growth	Inoculating loop, Ex. 4 Aseptic technique, Ex. 4 Plate streaking, Ex. 11 Graphing, App. D	1.0	0.5	
23 Physical Methods of Control: Ultra-violet Radiation	Basic	Use of ultraviolet light	Aseptic technique, Ex. 4	1.0	0.5	
24 Chemical Methods of Control: Disinfectants and Antiseptics	Basic	Modified use-dilution test	Inoculating loop, Ex. 4 Aseptic technique, Ex. 4 Pipetting, App. A	1.0	0.5	
25 Chemical Methods of Control: Antimicrobial Drugs	Basic	Disk-diffusion method	Inoculating loop, Ex. 4 Aseptic technique, Ex. 4	1.0	0.5	

Exercise Number and Title	Level	Techniques Introduced	Techniques Required	Time Required, in Hours		
				1st Lab Period	2nd Lab Period	3rd Lab Period
26 Effectiveness of Hand Scrubbing	Basic		Colony morphology, Ex. 3	1.0	0.5	
27 Regulation of Gene Expression	Enrichment	pGLO, nitrate reductase, anaerobic jar	Anaerobic culture techniques, Ex. 19 Nitrate reduction test, Ex. 17 Pipetting, App. A	1.0	0.5	
28 Isolation of Bacterial Mutants	Basic	Serial dilutions, replica plating	Spreading Rod, Ex. 27 Pipetting, App. A Serial dilution technique, App. B	0.5	1.5	1.0
29 Transformation of Bacteria	Basic	Minimal media	Aseptic technique, Ex. 4 Spreading rod, Ex. 27 Pipetting, App. A	1.5	0.25	
30 DNA Finger printing	Enrichment	Gel electrophoresis, RFLPs	Pipetting, App. A Electrophoresis, App. G	1.5		
31 Genetic Engineering	Advanced	Gel electrophoresis, gene splicing	Inoculating loop, Ex. 4 Spreading rod, Ex. 27 Pipetting, App. A Electrophoresis, App. G	1.5	1.5	0.5
32 Ames Test for Detecting Possible Chemical Carcinogens	Advanced	Ames spot test	Pipetting, App. A Aseptic technique, Ex. 10	1.5	0.5	
33 Fungi: Yeasts and Molds	Basic	Sabouraud agar for fungi, fungal slide culture	Compound light microscopy, Ex. 1 Wet mount, Ex. 2 Colony morphology, Ex. 3 Plate streaking, Ex. 11 Fermentation tests, Ex. 14 Dissecting microscope, App. E	1.0	1.0	
34 Phototrophs: Algae and Cyanobacteria	Basic		Compound light microscopy, Ex. 1 Hanging drop, Ex. 2	1.5		
35 Protozoa	Basic		Compound light microscopy, Ex. 1 Hanging drop, Ex. 2	1.5		
36 Parasitic Helminths	Basic	Estimating microscopic size	Compound light microscope, Ex. 1 Dissecting microscope, App. E	1.5		
37 Isolation and Titration of Bacteriophages	Enrichment	Serial dilutions, bacteriophage culture	Pour plate, Ex. 11 Pipetting, App. A Serial dilution technique, App. B Membrane filtration, App. F	0.25	1.5	0.25
38 Plant Viruses	Enrichment		None	0.5		
39 Epidemiology	Basic		Colony morphology, Ex. 3	0.5	0.25	

Exercise Number and Title	Level	Techniques Introduced	Techniques Required	Time Required, in Hours		
				1st Lab Period	2nd Lab Period	3rd Lab Period
40 Koch's Postulates	Advanced		Compound light microscopy, Ex. 1 Gram staining, Ex. 7 Plate streaking, Ex. 11 Starch hydrolysis, Ex. 13	1.0	0.5	0.25
41 Innate Immunity	Advanced	Lysozyme activity, bactericidin assay	Inoculating loop, Ex. 4 Pour plate, Ex. 11 Pipetting, App. A Serial dilution technique, App. B Spectrophotometry, App. C Graphing, App. D	1.5		
42 Agglutination Reactions: Slide Agglutination	Basic	ABO and Rh blood typing Bacterial identification	Dissecting microscope, App. E	0.5		
43 Agglutination Reactions: Microtiter Agglutination	Basic	Widal test for *Salmonella enterica*	Pipetting, App. A Serial dilution technique, App. B	1.0		
44 ELISA Technique	Advanced	ELISA technique	Pipetting, App. A Serial dilution technique, App. B Microtiter dilutions, Ex. 44	0.5	1.5	1.0
45 Bacteria of the Skin	Enrichment	Mannitol salt agar, coagulase test	Gram staining, Ex. 7 Plate streaking, Ex. 11 Selective media, Ex. 12 Fermentation tests, Ex. 14 Catalase test, Ex. 17	0.25	0.5	0.5
46 Bacteria of the Respiratory Tract	Enrichment	Blood agar	Gram staining, Ex. 7 Plate streaking, Ex. 11 Catalase test, Ex. 17 Kirby–Bauer technique, Ex. 25	0.25	1.0	
47 Bacteria of the Mouth	Enrichment	Blood agar, CO_2 incubation	Catalase test, Ex. 17 Dissecting microscopy, App. E	0.5	0.25	
48 Bacteria of the Gastrointestinal Tract	Enrichment	TSI, MacConkey agar, blood agar	Gram staining, Ex. 7 Plate streaking, Ex. 11 Fermentation tests, Ex. 14 Catalase test, Ex. 17 Blood agar, Ex. 46	0.25	0.5	0.5
49 Bacteria of the Genitourinary Tract	Enrichment	Calibrated loop, MacConkey agar, blood agar	Gram staining, Ex. 7 Plate streaking, Ex. 11 Fermentation tests, Ex. 14 Urea hydrolysis, Ex. 15 Oxidase test, Ex. 17 MacConkey agar, Ex. 48	0.5	1.0	0.5

Exercise Number and Title	Level	Techniques Introduced	Techniques Required	Time Required, in Hours		
				1st Lab Period	2nd Lab Period	3rd Lab Period
50 Identification of an Unknown from a Clinical Sample	Advanced		Gram staining, Ex. 7 Plate streaking, Ex. 11 Special media, Ex. 12 Kirby–Bauer technique, Ex. 25 Use of *Bergey's Manual,* Ex. 18		——Variable——	
51 Rapid Identification Methods	Enrichment	IMViC, Enterotube, API 20E	Inoculating loop and needle, Ex. 4 Aseptic technique, Ex. 4 Plate streaking, Ex. 11 MRVP tests, Ex. 14 Fermentation tests, Ex. 14 Protein catabolism, parts 1 and 2, Ex. 15 and 16 Respiration, Ex. 17	0.5	1.0	0.5
52 Microbes in Water: Multiple-Tube Technique	Basic	MPN for coliforms, MUG media	Aseptic technique, Ex. 4 Gram staining, Ex. 7 Plate streaking, Ex. 11 Special media, Ex. 12 Fermentation tests, Ex. 14 Pipetting, App. A	0.5	0.5	0.25
53 Microbes in Water: Membrane Filter Technique	Enrichment	Membrane filter techniques for fecal fecal coliforms and enterococci	Pipetting, App. A Membrane filtration, App. F	1.0	0.25	
54 Microbes in Food: Contamination	Basic	Standard plate count	Aseptic technique, Ex. 4 Pour plate, Ex. 11 Pipetting, App. A Serial dilution technique, App. B	1.0	0.25	
55 Microbes Used in the Production of Foods	Enrichment		Gram staining, Ex. 7 Plate streaking, Ex. 11 Pipetting, App. A	1.0	0.25	
56 Microbes in Soil: The Nitrogen and Sulfur Cycles	Advanced	Ammonification assay, nitrogen filtration, Winogradsky column	Compound light microscopy, Ex. 1 Wet mount, Ex. 2 Aseptic technique, Ex. 4 Simple staining, Ex. 5 Nitrate reduction test, Ex. 17	1.0	0.5	0.25
57 Microbes in Soil: Bioremediation	Enrichment	Hydrocarbon degradation	Compound light microscopy, Ex. 1 Gram staining, Ex. 7 Enrichment techniques, Ex. 12 Spreading rod, Ex. 27 Pipetting, App. A Serial dilution technique, App. B	1.0	1.0	

Techniques and Biochemical Tests, Alphabetical List