**SECTION A**

**ORGANIZATION**

This Instructor’s Handbook includes information that falls in three distinct categories: 1) a listing of equipment and supplies needed for performing all of the experiments, 2) procedures for setting up the experiments, and 3) answers to all the questions on the Laboratory Reports. These three categories of information have been designated as Sections A, B, and C, respectively.

**Section A** provides separate lists for equipment, supplies, cultures, and biologicals.

**Section B** attempts to identify any problems that might occur with the individual experiments to minimize experimental failures. No attempt is made to completely describe or justify each experiment in this section; only those problems that have been encountered with each experiment will be pointed out here. It is a good idea to read over each experiment before consulting this section to uncover any problems that might occur. Furthermore, alternative approaches and complementary procedures are suggested as enhancements for many of the exercises.

Section B also provides time allotments for each exercise. Each designated time is an approximation of the actual time required for the student in the laboratory. These times should be helpful in preparing a laboratory schedule. Although material lists are also given here for each exercise, *it is best to refer to the material lists in the manual at the beginning of each experiment when setting up the laboratory.*

**Section C** provides answers to the questions on the Laboratory Reports.

Basic Microbiology Laboratory Safety is reviewed in the manual on pages xi–xiii. Pages xiv–xvi contain a comprehensive list of microorganisms used and/or isolated in these exercises, along with their biosafety level and ATCC code. Instructors are strongly encouraged to study material safety data sheets (MSDS) for all reagents and organisms used in the laboratory, and to review the exercises with their institution’s biosafety committee.

**SECTION A**

**EQUIPMENT, SUPPLIES, AND CULTURES**

**A. Individual Student Supplies.** Each student should have a cabinet, drawer, or locker that contains the following items:

Bunsen burner

cover glass

eye protection

immersion oil

inoculating loop

inoculating straight wire

lab coat

labeling tape

lighter, flint

mechanical pipetting device

paper, bibulous

paper, lens

Sharpie® marking pen

slide holder (wooden clothespin)

slides, depression

slides, plain microscope

**B. General Items.** The following items should be available either at the student desk or at a nearby cabinet:

brightfield microscope

Bon Ami®

disinfectant for desktops

disposable latex/nitrile gloves

electric hot plates

Kimwipes®

metric rulers (small plastic)

scissors

sponges for disinfectant

staining kits (Simple, Gram, Spore, Acid-fast)

thermometer (0º–110ºC)

toothpicks (sterile)

Vaseline®

wash bottles

**C. Equipment.** Except for asterisked (\*) items, the following are used in experiments in these manuals. The asterisked items are either convenience items or preparation room equipment.

acid and base kits

antimicrobic disk dispensers

autoclave

\*automatic pipetting machine

balance, analytical

basins, surgical, stainless steel

blender, Waring

calibrated inoculating loops (.01 and .001)

cart, stainless steel

centrifuges

counters, hand tally type

\*digital video microscope

dishwasher, automatic

dissecting microscope

electrophoresis chambers and power supplies

filter assemblies, Millipore

fluorescent microscope

forceps

freezer (–20ºC and \*–80ºC)

GasPak jar

incubators

lamps, incandescent

lamps, ultraviolet

micropipettors (P10, P20, P200, P1000)

\*microwave oven

ocular micrometers

pH meter, electronic

phase-contrast microscopes

\*pipette washer, automatic

Quebec colony counters

refrigerators

slide rotator

spectrophotometer and cuvettes

stage micrometers

thermocycler

timers

UV transilluminator and \*photodocument system

vacuum pump with rubber hose

Vortex mixers

water baths, various sizes

\*water distiller or deionizer

**D. Miscellaneous Supply Items**

adhesive tape

aluminum foil

balloons

beakers, various sizes

bottles, glass

colony carriers

canisters for Petri dishes

canisters for used pipettes

centrifuge tubes and bottles

cotton, nonabsorbent

cotton, sterile, absorbent

disks, filter paper, ½" diameter

dissecting needles

dropping bottles, French square

Durham fermentation tubes

filter membranes, Millipore

filter paper, Whatman #2

flasks, Erlenmeyer, various sizes

GasPak envelopes and indicator strips

glass rods, stir, and L-shaped

graduates, all sizes

hand lens

hand lotion

microcentrifuge tubes, 1.7 ml

micrometers, ocular

micrometers, stage

micropipettor tips, sterile

mortars and pestles

Parafilm

PCR tubes, thin-walled 0.2 ml

Petri plates, glass

Petri plates, plastic

pH papers

pipettes, Pasteur with rubber bulbs

pipettes, serological (1, 5, and 10 ml)

racks, test tube, rubber coated

reference books for identification of microbes

rubber bands

rubber tubing for Bunsen burners

scalpels (sharp point)

soap, green

spot plates

stands, ring

sulfide test strips

swabs, sterile cotton

test tubes, various sizes

test tube caps

weigh paper and boats

**E. Chemicals.** In addition to general laboratory chemicals, this list includes the indicators, stains, and reagents from Appendix B and ingredients for special media found in Appendix C.

4-nitro-o-phenylenediamine

acetone

acid, acetic, glacial

acid, boric

acid, hydrochloric

acid, lactic

acid, molybdic

acid, sulfanilic

acid, sulfuric

agar

agarose

alcohol, ethyl

alcohol, isopropyl

alcohol, n-amyl

alpha naphthol

ammonium chloride

ammonium ferrous sulfate

ammonium oxalate

amphyl

ampicillin

auramine

barium chloride

basic fuchsin, 90% dye content

betadine

bleach

bromcresol green

bromcresol purple

bromothymol blue crystals

calcium carbonate

calcium chloride

calcium sulfate

casein, acid hydrolyzed

chloroform

cobalt nitrate

copper sulfate

cornstarch powder

cotton blue

cresol red

crystal violet, 85% dye content

cyclohexamide

dimethyl-α-naphthalamine

dimethyl-ρ-phenylenediamine hydrochloride

diphenylamine

dipotassium phosphate

disodium phosphate

EDTA

esculin

ferric chloride

ferrous sulfate

GelRed™ nucleic acid gel stain

gelatin

Glo Germ (Carolina Biological Supply)

glucose (dextrose)

glycerol

hydrogen peroxide

India ink

iodine crystals

lactose

Lysol

magnesium nitrate

magnesium chloride

magnesium sulfate

malachite green oxalate

mannitol

mercuric chloride

methyl red

methylene blue crystals, 90% dye content

molybdenum chloride

monopotassium phosphate

monosodium phosphate

nigrosin, water soluble

ρ-dimethylamine-benzaldehyde

paraffin

peptone

phenol crystals

phenol red

phenolphthalein

potassium chloride

potassium diphosphate

potassium hydroxide

potassium iodide

potassium nitrate

potassium permanganate

rhodamine B

safranin O

sodium acetate

sodium azide

sodium chloride

sodium citrate

sodium dodecyl sulfate (SDS)

sodium hippurate

sodium hydroxide

sodium lactate

sodium molybdate

sodium succinate

sodium sulfide

starch

streptomycin

sucrose

tannic acid

thymol blue

Tris

Tris-borate

tryptone

tryptophan, dl

Tween-80

vitamin B12

xylene cyanol FF

xylol

yeast extract

zinc chloride

zinc dust

zinc sulfate

**F. Biologicals**

Various antimicrobic sensitivity disks (high conc.)

API 20E test strips, bioMerieux, Inc.

API Staph-Ident test strips, bioMerieux, Inc.

bacitracin differential disks

Bio-Rad pGLO Bacterial Transformation kit

blood, defibrinated sheep

blood, synthetic (for typing)

blood typing antisera (anti-A, -B, and -D)

chloramphenicol

Difco Latex Test kit #3850-32-7

DNA ladder (1kb)

EnteroPluri-*Test* kits, Becton, Dickinson, and Company

ELISA, Edvotek: Aids Kit #1

Oxoid Diagnostic Streptococcal grouping kit consisting of latex reagents A, B, C, D, F, and G

PCR reagents (see Ex.65, Complete Version only)

plasma, rabbit, dehydrated

polyvalent Salmonella antiserum

polyvalent Shigella antiserum

pGLO plasmid DNA

RNase A

Salmonella “O” antigen, Group B

Salmonella “O” antiserum, poly A-1

*Salmonella typhi* “O” antigen

*Salmonella typhi* “O” antiserum

SXT sensitivity disks

Taxo P (Optochin) disks (BBL)

**G. Commercial Media.** Refer to Appendix C in both versions of the manual for lists of conventional media that are used in each book.

**H. Microbial Cultures.** For exercises other than unknowns. Asterisk (\*) indicates for Complete Version only.

*Alcaligenes faecalis*

*\*Bacillus cereus, var. mycoides,* ATCC 6462

*Bacillus coagulans*

*Bacillus megaterium*

*Bacillus subtilis*

*Candida glabrata*

*Chromobacterium violaceum*

*Clostridium beijerinckii,* ATCC 14950

*Clostridium sporogenes*

*Corynebacterium xerosis*

*Enterobacter aerogenes*

*Enterococcus faecalis*

*Escherichia coli,* ATCC 11775 Neotype

*Escherichia coli,* ATCC 8677 phage host

*Escherichia coli,* strain B, phage host

*Escherichia coli, HB101*

*Geobacillus stearothermophilus*

*Halobacterium salinarium*

*Klebsiella pneumoniae*

*Lactococcus lactis*

*Micrococcus luteus*

*Moraxella (Branhamella) catarrhalis*

*Mycobacterium smegmatis*

*\*Penicillium notatum*

*Proteus vulgaris*

*Pseudomonas aeruginosa*

*\*Pseudomonas fluorescens*

*Saccharomyces cerevisiae*

*Salmonella typhimurium*

*Serratia marcescens*

*Shigella flexneri*

*Sporosarcina ureae* ATCC 13881, Neotype

*Staphylococcus aureus*

*Streptococcus pyogenes*

Thermoanaerobacterium *thermosaccharolyticum*

\*Bacteriophage T4 ATCC 11303

**I. Bacterial Cultures for Unknowns**

*Alcaligenes faecalis* ATCC 8750, Neotype

*Bacillus polymyxis*

*Citrobacter freundii*

*Citrobacter intermedium,* biotype a

*Enterobacter cloacae*

*Enterococcus faecium*

*Lactobacillus acidophilus*

*Lactobacillus leichmanii*

*Micrococcus roseus* ATCC 186, Neotype

*Mycobacterium laticola*

*Neisseria mucosa*

*Staphylococcus epidermidis* ATCC 14990

*Staphylococcus saprophyticus*

*Streptococcus agalactiae*

*Streptococcus bovis (Enterococcus bovis)*

*Streptococcus dysagalactiae*

*Streptococcus mitis*

*Streptococcus mutans*

*Streptococcus pneumoniae*

*Streptococcus salvarius*

**J. Bacteriophage and Host Cells for Phage Typing.** Purchase from The American Type Culture Collection, **P.O. Box 1549** **Manassas, VA 20108**  
**(800) 638-6597,** http://www.atcc.org

27692 B1 CDC 52 I ATCC 27692

27700 B1 CDC 80 I ATCC 27700

27702 B1 CDC 3A II ATCC 27702

27696 B1 CDC 55 II ATCC 27696

27694 B1 CDC 55 III ATCC 27694

27706 B1 CDC 83A III ATCC 27706

27712 B1 CDC 42D IV ATCC 27712