**SECTION B**

**EQUIPMENT SETUP ASSISTANCE**

**LABORATORY PROTOCOL**

Although the students should be encouraged to read over this introductory material prior to the first laboratory period, the instructor will need to review certain aspects of it during the first meeting of the class. At this time the student will be informed about the scheduling of laboratory experiments, the location of various materials in the laboratory, the importance of not being late to class, the type of attire that is required, the importance of practicing aseptic techniques, and what to do about accidental spills. The instructor will undoubtedly mention additional “Rules of the Lab” that are not included here.

Certain materials, such as inoculating loops and Bunsen burners for microbial culturing or transfers, are used in many different exercises. In the “Materials” section for each exercise, reference will be made to the original exercise that describes the materials needed for a repeated procedure. For example, “Exercise 9: Aseptic Technique” will be referenced numerous times because it is essential to all experiments requiring microbial culturing, e.g., “Exercise 10: Pure Culture Techniques.” One can also assume that commonly used items, such as Sharpie marking pens for labeling plates and tubes, may be absent but implied on the material list.

CV refers to Benson Microbiology Laboratory Manual, **Complete Version**, 13/e.

SV refers to Benson Microbiology Laboratory Manual, **Short Version**, 13/e.

**PART 1**

**MICROSCOPY**

**Exercise 1**

**Brightfield Microscopy**

As in the case of all exercises in the manual, this exercise should be studied prior to discussion in the laboratory. This exercise describes operation and care of the brightfield microscope and prepares students for subsequent exercises that use the brightfield microscope to examine organisms.

***Materials:*** microscope slides, cover glass, immersion oil, lens tissue

Time Allotment: 30–60 min.

**Exercise 2**

**Darkfield Microscopy**

Since fluorescence microscopes use darkfield condensers, many instructors prefer to have their students read over this exercise before studying the fluorescent microscope. Studying motile bacteria with darkfield illumination can provide a worthwhile laboratory experience.

Time Allotment: 20–30 min.

**Exercise 3**

**Phase–Contrast Microscopy**

If student microscopes have phase–contrast optics incorporated into their regular (brightfield) scopes, this exercise will be performed right along with their studies of protozoa and algae. The study of protozoa provides a good opportunity to compare the two systems.

Time Allotment: 30–60 min.

**Exercise 4 (CV Only)**

**Fluorescence Microscopy**

The intent of this exercise is to provide the student with a theoretical, as well as a practical, understanding of a fluorescence microscope. If a student wishes to do an individual project that involves the use of this instrument, he or she should be able to proceed with relative safety if the outlined precautions are   
followed.

Demonstration Time: 5–60 min. Time depends on thoroughness of instruction.

**Exercise 5 (SV 4)**

**Microscopic Measurements**

In the morphological study of an unknown organism, as in Exercise 37 (SV 36), this technique is required. It may also be used for Exercises 6, 7, and 8 (SV 5, 6, and 7) when surveying the protozoa, algae, cyanobacteria, bacteria, and fungi.

Rather than allowing students to insert ocular micrometers in eyepieces, have some extra eyepieces with micrometers already installed in them that can be used in place of their regular eyepieces. This not only saves time, but it also lessens the possibility of damage to eyepieces.

***Materials:*** stage micrometers, ocular micrometers; see Ex. 1 for microscopy materials.

Time Allotment: 30–60 min.

**PART 2**

**SURVEY OF MICROORGANISMS**

**Exercise 6 (SV 5)**

**Microbiology of Pond Water—Protists, Algae, and Cyanobacteria**

Although cultures of protozoa and algae can be purchased from biological supply houses, the students will get much more out of these exercises if the specimens are collected from local ponds. Understandably, this is not always possible because of climatic conditions. The study of natural populations will reveal many ecological relationships that are not seen in cultures supplied commercially.

***Materials:*** bottles of cultures from ponds, rubber-bulbed pipettes, forceps (for filamentous algae), reference books; see Ex. 1 for microscopy materials.

Time Allotment: 3–4 hrs.

**Exercise 7 (SV 6)**

**Ubiquity of Bacteria**

This simple exercise introduces the student to the bacteria and their ubiquity in the environment. Soil plates can be prepared by students and used in Ex. 16 (SV 15) for the endospore stain as they usually provide better results than stock cultures.

***Materials:*** nutrient broth tubes, blood agar plates, trypticase soy agar plates, sterile swabs, Sharpie marking pen; see Ex. 11 (SV 10) for smear preparation materials, Ex. 12 (SV 11) for simple stain materials, and Ex. 1 for microscopy materials.

Time Allotment: First Period—30 min.

Second Period—45 min.

**Exercise 8 (SV 7)**

**The Fungi: Yeasts and Molds**

Exposure of Petri plates of Sabouraud’s agar to air for 30–60 minutes. Experimentation will be necessary to determine what is optimal for different parts of the country and how seasons affect numbers. If air collection does not work well, one can inoculate plates of different kinds of molds that students can use for making their own slides. The plates should be incubated at room temperature for 4–7 days prior to study.

It is strongly recommended that Exercise 24 (SV 22) be combined with this exercise so that students make slide cultures of the various types of molds. Identification of molds is very difficult and time-consuming when wet mounts are made directly from Petri plates. Although making a slide culture delays completion of the experiment by one laboratory period, it is not necessarily more time-consuming. A slide culture can be made while the protozoans and algae are being studied so that once the algae are finished, the slide culture is ready for staining. Incidentally, the cotton blue (Poirrier’s blue) for the lactophenol cotton blue stain can be purchased from Fisher Scientific, St. Louis, Missouri. This is an excellent mounting medium for mold hyphae.

The cellophane tape method works very well for observing fruiting bodies, but the tape must be clear. When students are finished, the tape should be disposed of properly and autoclaved.

***Materials:*** prepared slides and broth cultures of *Saccharomyces cerevisiae,* mold cultures (air contaminants) on Sabouraud’s agar, sharp-pointed scalpels, dissecting needles, lactophenol cotton blue stain; see Ex. 1 for microscopy materials. Clear cellophane tape.

Time Allotment: 2–3 hrs.

**PART 3**

**MANIPULATION OF MICROORGANISMS**

**Exercise 9 (SV 8)**

**Aseptic Technique**

It is essential that all students master aseptic technique, but with beginning students this will take time and practice. The obvious mistakes will be made by students: 1) not using the small finger to remove caps from tubes; 2) placing caps and Petri plate lids on the bench top while manipulating cultures; 3) only flaming the loop and not the entire wire; and 4) gouging the agar surface during inoculation. Students will have completed Ex. 7 (SV 6), so they should have an appreciation of the potential for contamination if correct procedures are not carried out.

***Materials:*** materials for microbial transfers include inoculating loop, Bunsen burner, lighter, marking pen, disinfectant and sponge; additional materials for this exercise include nutrient agar slants, nutrient broth tubes, cultures (slant, broth, and plate) of *E. coli*.

Time Allotment: First Period—1 hr.

Second Period—30 min.

**Exercise 10 (SV 9)**

**Pure Culture Techniques**

To ensure that students master pure culture techniques, we have this exercise as a practicum in which students must show a successful streak plate with isolated colonies. They are allowed to repeat the streak plate if they are not successful the first time. It may be helpful for beginners to draw lines with a marking pen on the plate to delineate quadrants. A reminder to label the base of the plate and not the lid is usually required. *S.* *marcescens* must be incubated at room temperature for red color to develop. A 2:1 mixture of *S. aureus* and *E. coli* overnight cultures, which is then diluted 2 to 3 fold (so that *E. coli* does not overgrow *S. aureus*), also works well. Likewise, other combinations of bacteria work well for this experiment; however, the instructor should become familiar with the growth rates and colony characteristics of each individual species before attempting to mix species. Also, using microscopically distinct bacteria helps with further evaluation of colonies and slant purity.

Students tend to make several mistakes in their first attempt at preparing streak plates: 1) the agar is gouged; 2) the entire plate surface is not used when streaking; 3) too many or too few streaks per quadrant; and 4) the plates are not inverted when incubated. If students are required to pour plates for streaking, they must be reminded to pour the media into the plate bottom and not the lid and not to wait too long before pouring or else the agar will solidify in the tube.

***Materials:*** nutrient agar pours, sterile Petri plates, nutrient agar slants, 50ºC water baths (thermometer, beaker with burner, tripod, and wire gauze or electric hotplate), nutrient broth mixed cultures of *S. marcescens* or *E. coli* and *M. luteus* or *C. violaceum;* see Ex. 9 (SV 8) for aseptic culturing materials, Ex. 11 (SV 10) for smear preparation materials, and Ex. 15 (SV 14) for Gram stain materials.

Time Allotment: First Period—1 hr.

Second Period—1 hr.

Third Period—30 min.

**PART 4**

**STAINING AND OBSERVATION OF MICROORGANISMS**

**Use of Pathogens:** The suggestion has been made by some correspondents that *P. aeruginosa* and *S. aureus* should be replaced by nonpathogens. If one feels insecure in using these organisms, *Proteus vulgaris* and *Micrococcus luteus* can be substituted with satisfactory results. We have not experienced any problems with these organisms in many years of teaching.

**Exercise 11 (SV 10)**

**Smear Preparation**

The smears made during this period will be used in “Ex. 12 (SV 11): Simple Staining” and “Ex. 14 (SV 13): Capsular Staining.” The only problem encountered with this exercise is preventing the students from making a smear with excessive amounts of bacteria, particularly when obtaining the specimen from solid media. A simple reminder to students that visible bacterial colonies may contain millions of cells should be helpful.

***Materials:*** materials for smear preparation include microscope slides, slide holder (clothespin), inoculating straight wire and loop, Bunsen burner or incinerator, marking pen; see Ex. 12 (SV 11) and Ex. 14 (SV 13) for bacterial cultures.

Time Allotment: 30 min.

**Exercise 12 (SV 11)**

**Simple Staining**

No problems are typically encountered. We have substituted *Corynebacterium xerosis* for the avirulent strain of *C. diphtheria.* This organism should still allow students to do a useful morphological study with a simple procedure.

***Materials:*** nutrient agar slant culture of avirulent *C. xerosis*, Loeffler’s methylene blue, wash bottle, bibulous paper; see Ex. 11 (SV 10) for smear preparation materials and Ex. 1 for microscopy materials.

Time Allotment: 30 min.

**Exercise 13 (SV 12)**

**Negative Staining**

Two methods are provided here for making a slide. Wayne Merkley at Drake University suggested the second method, indicating that his students get better results with this method.

Whichever technique is used, students should look for molds and yeasts as well as bacteria of different types. Spirochaetes may also be present.

***Materials:*** nutrient agar slant cultures of *S. aureus* and *B. megaterium,* inoculating straight wire and loop, Bunsen burner, lighter, Sharpie pen, felt-tip marking pen, microscope slides (with polished edges), nigrosin solution or India ink, sterile toothpicks; see Ex. 1 for microscopy materials.

Time Allotment: 30 min.

**Exercise 14 (SV 13)**

**Capsular Staining**

This is the Anthony method for staining capsules. It is critical that smears not be heat-fixed for the stain to succeed. The air-dried smear is first stained with 1% crystal violet followed by a wash with 20% copper sulfate. The cells stain deep blue/purple with light blue capsules. Appropriate waste basins should be provided for the copper sulfate wash as copper sulfate should not be discharged into sinks. Waste copper sulfate should be disposed of according to safety rules at your respective institutions. Also provide a disinfectant bath for used microscope slides. Negative staining with nigrosin can also be used to demonstrate capsules.

***Materials:*** 36–48-hour milk culture of *Klebsiella pneumoniae,* 1% crystal violet, 20% copper sulfate in small wash bottles or stain bottles; see Ex. 11 (SV 10) for smear preparation material and Ex. 1 for microscopy materials. Nigrosin.

Time Allotment: 30 min.

**Exercise 15 (SV 14)**