GENERAL INFORMATION

The purpose of this laboratory course is to introduce you to a sampling of the morphologically and physiologically diverse members of the Prokaryotes. The emphasis is on the enrichment, purification, and identification of organisms taken from natural habitats, but we will also explore genetic phenomena using model bacteria. The Bacteria and Archaea domains are so vast and diverse that you can study only a miniscule portion of the organisms and their isolation techniques in a one-term course. We will not study, fungi, algae, protozoans, slime molds, nor a number of other microbial groups. Each deserves its own course.

This manual was originally written by Bill Sistrom and Dick Castenholz. Additions and modifications have been made by other contributors.

Format

A lecture for the laboratory is scheduled for 9:00-9:50 am on Mondays. In the lecture background information and technical suggestions will be given. Though attendance won't be taken, you are expected to attend these lectures so that you will be prepared when you arrive at your lab. The goal with the Monday lectures is to maximize your time working in the lab, and so introductions and overviews won't routinely be given in the lab. If you miss the lectures you will likely be unprepared to perform that week's exercises.

Laboratories meet twice each week for up to 2 hours each session. Many sessions will not last the full time, though during some weeks you will have additional lab work outside of your normal section time. The laboratory will be open about 8-5 daily. You may work in the lab at any time except when there is another class in session. However, you must attend your regularly scheduled lab section.

You will need a notebook in addition to this manual for this course. The notebook can be of any type that suits you. The idea is that you will take detailed notes about your results and observations throughout the term.

We will do two types of exercises: general exercises and enrichments or isolations. The former will be done by all students at the same time, and are designed to introduce you to some of the commonly used techniques in the many branches of microbiology. These will include three larger scale experiments which will take from 1 to 4 weeks each. The enrichment exercises are for the concentration or isolation of specific groups of microorganisms from mixed populations; all students will perform these, but once begun, the enrichments won't require coordinated efforts by the entire class. Nonetheless, you are expected to arrive on time to each session. We will begin most sessions with a brief introduction about the work to be done that day, and then commence with the general exercises or enrichments.

Assignments and grading
Grades will be assigned on points earned out of a possible 320 based upon the following criteria:

Lab quizzes (80 points [40 points each]). There will be 2 quizzes during lab lecture. These are intended to test your knowledge of the techniques we use and of the physiological, ecological, and biochemical characteristics of the organisms that we study. No makeup quizzes will be given unless prior arrangements are made, or a valid medical or travel excuse is provided.

Mid-term lab practical (60 points). During the 5th week you will take a lab practical exam. Grading will be based upon your ability to perform some of the standard microbiological techniques, and to analyze results from isolations and tests that were previously done in the lab.

Worksheets (50 points [10 points each; lowest score dropped]). Short worksheets will be required for 6 of the general exercises. Due dates will be listed in the schedule.

Identification of 2 unknowns (30 points [10 points each; 10 points for key]). You will be given a mixture of 2 species from bacterial groups that we will have studied, and your task will be to purify and identify them to the genus or species level based upon an identification key of your design. You will submit a copy of your key during week 6 when you receive your unknowns. You will be given a form with which to report your identifications, and it will be due during the last lab.

Term paper (80 points). You and your lab partner will choose one of three large lab projects to write about as a journal styled paper. Details will be provided during the term.

Discretionary (20 points). This will be based upon participation, group cooperation, workstation cleanup, etc.

Attendance. You are expected to attend all labs. If you are more than 15 minutes late for your lab, you will be counted as absent. One absence will be excused, but additional absences will incur a penalty of 10 points per absence. You will be allowed to attend a different section to make up one absence during the term, and this must be arranged in advance. However, many of the projects are done in groups, and you will thus be responsible for completing those projects with those group members.

There are 47 scheduled contact hours for this course, and you will need to spend additional time in the lab to continue several experiments. This generally requires 1-3 additional hours per week beginning in week 4. However, no additional time should be needed during weeks 5 and 10.

Safety

Most of the organisms with which we will work are non-pathogenic. However, you should take precautions that you would use as if you are working with known pathogens—one is never completely certain what an enrichment will yield from a heterogeneous source. Wash your hands frequently, don't put your hands to your face when handling materials, and don't pipette by mouth. The chemicals and stains that we routinely use in the microbiology laboratory are not particularly toxic, but, again, use due caution. Lab coats are not required, though you are welcome to use one or some other such covering while working in the lab. Invariably there are spills of stains, and these will permanently stain clothes. Absolutely NO FOOD OR DRINK is allowed in the lab.

In some cases we will be using organisms that are considered to be opportunistic pathogens (members of the normal microflora that are *capable* of initiating an infectious process but normally do not). In those exercises you will be given specific instructions for their handling, which include but are not limited to the use of gloves, wiping down lab benches with disinfectant at the end of the exercise, and disposal of all supplies that come in contact with the organisms into autoclave bags.

Perhaps the greatest constant danger in the microbiology lab is the use of gas (Bunsen) burners. You will have burners in front of you, on at least one side of you, and likely behind you as well. Long hair should be tied up or back, and clothing should not be excessively loose.

The workspace

Common supplies

Stocks of microscope slides, coverslips, Kimwipes, lens paper, and bibulous paper will be kept in the lab. Also, sterile, disposable Pasteur pipettes will be kept in large stoppered test tubes; do not put used pipettes back into these tubes! Small, capped test tubes of sterile fresh water and salt water are regularly stocked. These can be used for a variety of purposes. Please do not return these to the common rack after you have used them, because we can't insure that they are sterile.

Common spaces

Please keep the common spaces clean and organized; these include some benches, all incubators, and lab shelves. Don't leave old cultures behind—it is your responsibility to dispose of them properly when your exercises and enrichments have been completed. Wipe up spills, clean common tools and equipment, put away your source materials, and remove labels from tubes, caps, and flasks.

Culture disposal

Most plates will be disposed of in the large metal trash barrel, which is emptied every night. When we are working with potentially pathogenic organisms, plates will go in the white autoclave buckets. Liquid cultures can be poured down the sink unless otherwise directed; cultures of potentially pathogenic organisms will be autoclave or bleached.

Glass

Under no circumstances should glass be put in the normal trash. Microscope slides and coverslips, disposable Pasteur pipettes, broken tubes, and other broken glassware must be put in the special bucket designated for glass.

Your bench

Though you will work at the same bench throughout the term, you will be sharing the space and tools with several students who are in other sections. It is important that you keep your workspace neat and clean, and that you keep track of your tools. If something is missing please ask your instructor or TA to find a replacement. "Borrowing" from an unoccupied bench means that the students who work at that space in another section will be missing some of their tools or supplies.

Each bench should have a small dropper bottle of immersion oil for microscopy. These bottles invariably leak if they are in any position but upright, so keep the oil bottle either on your bench top or alone in a small container in your tool tray.

Microscopes

You will be assigned a microscope that you will use throughout the term. Three other students (in the three other lab sections) will use the same microscope. You will not have access to other microscopes, nor will any other students have access to yours. They will be kept in locked cabinets, and you will be provided with a key for that cabinet; there is a $5 deposit for the key that will be refunded when you return the key at the end of the term. If you come to the lab during open hours without your key, you will not have access to your microscope. We instituted this policy in response to the theft of a microscope during a recent term.

Labeling

You will use dozens of tubes, flasks, and Petri dishes throughout the term, and keeping track your cultures and enrichments will be crucial. Use fine point "Sharpies" and write on tape for the labeling of all flasks, bottles, and tubes (use the colored tape rather than masking tape). Always include your initials and lab section (I, II, III, or IV). Do not label plastic or metal caps of tubes or flasks, and never label the frosted glass area on a flask or tube. Always include the source of material whenever appropriate.

Label Petri dishes on the bottom (the part with the agar). Don't use tape for the labels, as the tape will prevent you from observing the agar from the bottom side. Keep labels as small as possible (don't try to include the whole history of the culture—do that in your notebook). Remember, you'll want to look at the colonies, and this is hard to do against a heavily labeled background. Petri plates with agar are kept in the inverted position with the agar side up. This prevents condensation from forming on the cover, which can drip onto the colonies on the agar causing cells to spread into films rather than remain in isolated colonies.