

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.

Format

Microbiology (Bi330) is a required pre- or co-requisite for *Microbiology Lab*. If a student withdraws from Bi330, they must withdraw from Bi331. Students failing to do so will be dropped from Bi331 upon our request to the Registrar.

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 weeks exercises.

Laboratories meet twice each week for up to 2½ 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, Monday through Friday. 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 begin most sessions with a brief introduction about the work to be done that day, and then commence with the general exercises or enrichments. We will do three types of exercises: general exercises, enrichments, and projects. General exercises 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. The enrichments 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. The projects are larger scale experiments that will take from 1 to 4 weeks each to complete; three of these are group (2-4 students) projects that, once begun, will require group members to coordinate daily or weekly tasks.

Assignments and grading

Grades will be assigned on points earned out of a possible 400 based upon the following criteria:

5-minute quizzes (55 points [5 points each]). There will be a short quiz at the beginning of 12 of the labs in which new exercises will be performed (except for the first lab). The questions will cover the procedures and background found for those exercises in the lab manual. The purpose of these quizzes is to motivate you to read the material before arriving in lab. The low score will be dropped.

Lab exams (100 points [50 points each]). There will be 2 exams 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 exams will be given unless prior arrangements are made, or a valid medical or travel excuse is provided.

Lab practical final exam (70 points). During the 10th 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 (85 points [10 points each; one at 5 points]). Short worksheets will be required for 9 of the general exercises. Due dates will be listed in the schedule.

Identification of 2 unknowns (40 points [15 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 species level based upon an identification key of your design. You will submit a copy of your key during week 5 when you receive your unknowns.

Abstract (40 points). You will write an abstract for one of three lab projects (Genetic analysis of prodigiosin biosynthesis; The nitrogen cycle in a biological filter; Culture-independent methods for studying microbial communities). The text of each of your abstracts must be typed and double-spaced, in 12 pt. font, be no more than two pages, and must include:

- A statement of the goal of the experiment in the context of relevant background information.
- The methods, **without procedural details** (such as dilutions, reagents, standards, etc.), employed to address the question.
- A summary of the results you obtained. You should include a table, relevant figure, graph, and/or statistical analysis, but provide these on one separate page.
- Analysis of the results and conclusions that you can draw from them

For examples refer to the abstract of any article in an American Society for Microbiology publication, such as the *Journal of Bacteriology*. However, note that abstracts in those journals do not include tables/figures/graphs.

Discretionary (10 points). This will be based upon participation, group cooperation, workstation cleanup, punctuality, 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.

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 we recommend that you use one or some other such covering while working in the lab. Invariably there are spills of stains, and these will permanently stain clothes. Open-toed shoes are not allowed in the lab. Absolutely NO FOOD, DRINK, OR GUM is allowed in the lab. Cell phones must be kept out of reach at all times.

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. 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 trash barrels. 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 have access to no 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. You must return the key at the end of the term; if you fail to do so, your UO account will be charged (the current replacement cost is around \$22!). 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.

Microbiology Lab, Spring 2016

Schedule

Week	Lab*	Exercise	Page [†]
1 3/28-3/31	1	GE1: Airborne microbes GE2: Simulated epidemic GE3: Streak dilution technique	5 6-7 9-13
	2	GE4: Microscope use GE8: Serial dilution for bacterial enumeration	14-17 22-26
2 4/4-4/7	1	Enrichment for <i>Caulobacter</i> GE9: Constructing a growth curve for <i>E. coli</i>	63-64 27-30
	2	The nitrogen cycle in a biological filter GE6: Staining methods (Gram)	71-74 Belser and Mays 20
3 4/11-4/14	1	GE14: Ultraviolet light-induced kill rate GE12: Identification of Gram-positive cocci	53-58 41-47
	2	Enrichment for denitrifying bacteria Enrichment for aerobic N ₂ -fixing bacteria Enrichment for anaerobic N ₂ -fixing bacteria	75 76-77 77
4 4/18-4/21	1	GE6: Staining methods (endospore) Receive unknowns Genetic analysis of prodigiosin biosynthesis	21 83-87 65-68 Morrison
	2	GE6: Staining methods (capsule) GE13: Testing sensitivity to antibiotics	20-21 49-52
5 4/25-4/28	1	GE10: MPN method for coliform counts	31-36
	2	Enrichment for Cyanobacteria Enrichment for Purple non-sulfur bacteria	79-80 81-82
6 5/2-5/5	1	Complementation analysis of prodigiosin mutants	69-70
	2	Bacterial motility GE6: Staining methods (flagella)	Handout 21
7 5/9-5/12	1	GE15: Bacterial populations of the skin	59-61
	2	Culture-independent methods for studying microbial communities	Handout Specter
8 5/16-5/19	1	TBA	
	2	GE11: Membrane filter method for bacterial counts	37-40
9 5/23-5/26	1	No new projects	
	2	Lab clean up and <i>Microbial Jeopardy</i>	
10 5/30-6/2	1	Monday: Memorial day—no lab Tuesday: Lab Practical—no open lab	
	2	Lab Practical—no open lab	

* Lab 1 is on Monday and Tuesday; Lab 2 is on Wednesday and Thursday

[†] Page numbers are from the lab manual; authored articles are posted on Canvas.

Exam and Assignment Due Dates

Week	Assignment	Date
2	GE2 worksheet (Simulated epidemic)	Mon. 4/4 (M/W labs) Tues. 4/5 (T/Th labs)
2	GE8 worksheet (Serial dilution for bacterial enumeration)	Wed. 4/6 (M/W labs) Thurs. 4/7 (T/Th labs)
3	GE9 worksheet (Constructing a growth curve for <i>E. coli</i>)	Mon. 4/11 (M labs) Tues. 4/12 (T labs)
4	GE14 worksheet (Ultraviolet light-induced kill rate)	Mon. 4/18 (M labs) Tues. 4/19 (T labs)
5	Exam 1 (in lab lecture, 123 PAC)	Mon. 4/25
5	Identification key for unknowns	Mon. 4/25 (M/W labs) Tues. 4/26 (T/Th labs)
5	GE13 worksheet (Antimicrobial agents)	Wed. 4/27 (M/W labs) Thurs. 4/28 (T/Th labs)
6	GE10 (MPN method for coliform counts) worksheet	Wed. 5/4 (M/W labs) Thurs. 5/5 (T/Th labs)
8	GE12 (Identification of Gram-positive cocci) worksheet	Wed. 5/11 (M/W labs) Thurs. 5/12 (T/Th labs)
9	GE15 (Bacterial populations of the skin) worksheet	Mon. 5/23 (M/W labs) Tues. 5/24 (T/Th labs)
9	GE11 (Membrane filter method for bacterial counts) Unknowns worksheet	Wed. 5/25 (M/W labs) Thurs. 5/26 (T/Th labs)
10	Abstracts	Tues. 5/31 (M/W labs) Wed. 6/1 (T/Th labs)
10	Practical exams	Tues. 5/31 (T/Th labs) Wed. 6/1 (M/W labs) Thurs. 6/2 (T/Th labs) Fri. 6/3 (M/W labs)
Finals	Exam 2 (in 123 PAC) 10:15	Mon. 6/6 (all labs)