Bi331 Microbiology Lab Syllabus

Spring 2022

TENTATIVE SCHEDULE OF LECTURES AND LAB ACTIVITIES

Week	Lab*	Exercise	Page†
1 3/28-3/31	**Lecture 1**	**General lab/class business and introduction to week 1 general excercises**	
	1	GE1: Airborne microorganisms GE2: Simulated epidemic GE3: Streak dilution technique GE4: Intro to Microscope use	7 8-9 13-16 17-20
	2	GE4: Microscope use GE5: Micropipetting and serial dilutions	17-20 21-24
2 4/4-4/7	**Lecture 2**	**Bacterial growth curves, Gram stain, nitrogen cycle**	
	1	GE6: Constructing a growth curve for *E. coli* P1: Look up unknowns and work on key	27-28 72-76
	2	GE7: Staining methods (Gram) P2: The nitrogen cycle in wastewater treatment	31 79-83 Belser and Mays
3 4/11-4/14	**Lecture 3**	**UV irradiation, Gram+ cocci, endospore stain, nitrogen cycle continued**	
	1	GE8: Ultraviolet light-induced kill rate GE9: Identification of Gram-positive cocci	33-36 39-44
	2	GE7: Staining methods (endospore) EN1: Enrichment for denitrifying bacteria	32 67
4 4/18-4/21	**Lecture 4**	**Prodigiosin biosynthesis, capsule stain, antimicrobial agents**	
	1	P1: Receive unknowns P3: Genetic analysis of prodigiosin biosynthesis	72-76 84-87 Morrison
	2	GE7: Staining methods (capsule) GE10: Testing sensitivity to antibiotics	31-32 47-49
5 4/25-4/28	**Lecture 5**	**Exam 1 – taken during lecture period**	
	1	Continuation of existing exercises	
	2	GE7: Staining methods (flagella) GE11: Membrane filter method for bacterial counts	32 53-54
6 5/2-5/5	**Lecture 6**	**Cyanobacteria and purple non-sulfur bacteria**	
	1	P3: Complementation analysis of prodigiosin mutants	88-89
	2	EN2: Enrichment for Cyanobacteria EN3: Enrichment for Purple non-sulfur bacteria	68-69 70-71
7 5/9-5/12	**Lecture 7**	**Skin bacteria**	
	1	GE12: Bacterial populations of the skin	57-58
	2	Continuation of existing exercises	
8 5/16-5/19	**Lecture 8**	**Most Probable Number technique**	
	1	GE13: MPN method for coliform counts	61-64
	2	Continuation of existing exercises and *Microbial Jeopardy*	
9 5/23-5/26	**Lecture 9**	**Extra Molly office hours/turn in worksheets**	
	1	Lab Practical – no office hours/no open lab time	
	2	Lab Practical – no office hours/no open lab time	
10 5/30-6/2	**Lecture 10**	**Monday: Memorial Day – no lecture**	
	1	No lab Monday and Tuesday	
	2	Exam 2 – taken during lab section	

Lectures are on Mondays at 11:00-11:50 am in McKenzie Hall room 129

* 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: Simulated Epidemic Worksheet	Mon. 4/4 (M/W labs) Tues. 4/5 (T/Th labs)
2	GE5:Serial Dilution Worksheet	Wed. 4/6 (M/W labs) Thurs. 4/7 (T/Th labs)
3	GE6: Growth Curve Worksheet	Mon. 4/11 (M/W labs) Tues. 4/12 (T/Th labs)
4	GE8: Ultraviolet Light-Induced Kill Rate Worksheet P1: Identification Key for Unknowns	Mon. 4/18 (M/W labs) Tues. 4/19 (T/Th labs)
5	Exam 1 (during lecture period, MCK 129)	Mon. 4/25 (lecture)
5	GE10: Antibiotic Sensitivity Worksheet	Wed. 4/27 (M/W labs) Thurs. 4/28 (T/Th labs)
6	GE11: Membrane Filtration Worksheet	Wed. 5/4 (M/W labs) Thurs. 5/5 (T/Th labs)
8	GE9:Gram-positive Cocci Identfication Worksheet	Wed. 5/18 (M/W labs) Thurs. 5/19 (T/Th labs)
9	GE12: Bacteria of the Skin Worksheet GE13 MPN Worksheet P1: Unknowns Identification Worksheet	Mon. 5/23 (lecture)
9	Practical Exam (during lab, Klamath 33, sign-up for time)	Mon. 5/23 (M/W labs) Tues. 5/24 (T/Th labs) Wed. 5/25 (M/W labs) Thurs. 5/26 (T/Th labs)
9	P2 or P3: Summaries (uploaded to canvas by 3:00 pm)	Fri. 5-27 (by 3:00 pm)
10	Exam 2 (during lab section, Klamath 33)	Wed. 6/1 (W labs) Thurs. 6/2 (Th labs)

CLASS SCHEDULE

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
8:00 AM					
			Bi330 LECTURE Dr. Alice Naftaly MCK 129 8:30-9:50 AM		Bi330 LECTURE Dr. Alice Naftaly MCK 129 8:30-9:50 AM
9:00 AM					
10:00 AM	OFFICE HOURS Rob Porch MCK 471 10:00-11:00 AM		OFFICE HOURS Emily Hill Klamath 33 10:00-11:00 AM	OFFICE HOURS Patrick Horve Klamath 33 10:00-11:00 AM	OFFICE HOURS Dr. Alice Naftaly MCK 129 10:00-11:00 AM
11:00 AM	Bi331 LECTURE Dr. Molly Jud MCK 129 11:00-11:50 AM	Bi331 LAB 1 SECTION III Klamath 33 11:00AM-1:20PM		Bi331 LAB 2 SECTION III Klamath 33 11:00AM-1:20PM	
12:00 PM					
1:00 PM	Bi331 LAB 1 SECTION I Klamath 33 1:00-3:20 PM		Bi331 LAB 2 SECTION 1 Klamath 33 1:00-3:20 PM		Bi331 Lab Prep Klamath 33 1:00-3:00 PM Staff-only
		OFFICE HOURS Dr. Molly Jud Klamath 33 1:20-2:20 PM			
2:00 PM					
3:00 PM		Bi331 LAB 1 SECTION IV Klamath 33 3:00-5:20 AM		Bi331 LAB 2 SECTION IV Klamath 33 3:00-5:20 AM	
4:00 PM	Bi331 LAB 1 SECTION II Klamath 33 4:00-6:20 PM		Bi331 LAB 2 SECTION II Klamath 33 4:00-6:20 PM		
5:00 PM					
6:00 PM					
7:00 PM					
8:00 PM	OFFICE HOURS Maddie McAndrew Zoom 8:00-9:00 PM				

You may work in the lab at any time, except when there is another class in session (ie-all gray boxed times are unavailable for out-of-class-time work).

GENERAL INFORMATION

Objectives

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.

- **Enrich, purify, identify, and explore genetic systems of the diverse Prokaryote group**
  - wild strains (from natural habitats)
  - lab strains (used as model systems)
- **Learn standard lab and microbiology techniques**
  - sterile technique, bacterial husbandry, microscopes, etc
- **Learn to follow a protocol**
  - follow directions (written and verbal)
  - deal with clean-up, hazards, safety
- **Exposure to real-life lab work scenarios and experience**
  - perform actual experiments
  - juggle multiple projects and experiments at once
  - gain independence in lab

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 11:00-11:50 am on Mondays in McKenzie Hall 129. In the lecture, background information and technical suggestions will be given. Attendance will be taken via iClicker; this is to help incentivise you 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 the exercises for those weeks.

Laboratories meet twice each week for up to 2 hours and 20 minutes each session in Klamath 33. Many sessions will not last the full time, though during some weeks you will have additional lab work outside of your normal section time. You will have access via your UO ID card to the laboratory from 8:00am-9:00pm Monday through Friday, and 8:00am-6:00pm on Saturday and Sunday. A buddy-system will be mandatory for working in the lab after hours (6:00pm-9:00pm) and on the weekends (see description of the buddy-system on page 5). You may work in the lab at any time except when there is another class in session (see class schedule on page iv). 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 lab exercises. We will do three types of exercises: general exercises (GEs), enrichments (ENs), and projects (Ps). 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 6 weeks each to complete; some 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 ***450*** based upon the following criteria:

5-minute Quizzes (70 points [most are 5 points each]). There will be a short quiz, to be taken on canvas prior to your lab, on 13 of the labs in which new exercises will be performed. The first quiz will be 15 points to be taken prior to your first lecture; the remaining 12 quizzes will be 5 points each and taken before each lab (due 30 minutes before your designated lab section). 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. All quizzes are open note (you may use the lab manual to find answers); however, you may not use any other sources. Since there is a time limit on these quizzes, it is highly suggested to read to lab manual first before starting your timed quiz. The low score of one of the twelve 5-point quizzes will be dropped (total points = 15+[11*5] = 70 points).

Lab Exams (100 points [50 points each]). There will be 2 closed-note exams: one during lab lecture and one during your lab section. 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 (75 points). During the 9^{th} 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. Office hours will be cancelled during week 9 due to grading for the practical exam.

Worksheets (85 points [10 points each; one at 5 points]). Short worksheets for general experiments will be required for 9 of the general exercises. Due dates will be listed in the schedule (see page iii). Sloppy work will be penalized. Calculations must be clear, graphs must be constructed via computer programs or done on graph paper, mistakes must be erased, and multiple pages must be stapled.

Identification of 2 Unknowns (50 points [15 points each; 20 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 4 when you receive your unknowns.

Summary (50 points). You will write a summary for one of two lab projects (P2:The nitrogen cycle in a biological filter or P3: Genetic analysis of prodigiosin biosynthesis). The text of the summary must be typed and double-spaced, in 12 point font, 1-inch margins, be no more than two pages, and must include:

- A statement of the goal of the experiment in the context of relevant background information (cite the papers available on canvas).
- 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 the them

Attendance/Discretionary (15 points). This will be based upon attendance (iClicker during lectures), participation, group cooperation, workstation cleanup, punctuality, etc. If you forget your clicker or it malfunctions during lecture, do not ask your instructor for attendance points for that day. It is your responsibility to remember your clicker and keep it in good working order (keep batteries on hand). You will be permitted to miss one lecture without it impacting your grade. All lectures will be in-person-only; there will be no video or zoom options. If you have to miss a lecture for any reason, make sure to look over the lecture slides (posted to canvas), borrow notes from your peers, and come to office hours to get up to speed.

Special note about lab attendance. You are expected to attend all labs. In the past, points have been deducted for absences or being late to lab. However, to help promote responsible behavior in regards to COVID, you will not be punished for missing labs and absences due to COVID will be considered excused absences. This only applies if you have been exposed to a known COVID-positive person or have tested positive yourself and need to quarentine. YOU MUST NOTIFY YOUR INSTRUCTOR VIA E-MAIL AT LEAST 2 HOURS PRIOR TO THE LAB SECTION(S) YOU WILL MISS. Your instructor will work with you to ascertain a plan for making up the missed lab work. Since quizzes are all done virtually through Canvas, you will still be responsible for these items (see Quizzes above). If you do not notify your instructor of your absence as described above or are more than 10 minutes late for your lab, you will be counted as absent. If you are absent for more than 3 unexcused sessions you will receive a failing mark for the course.

Student Experience Survey (5 points). At the end of the course, please fill out the student experience survey. This is an important professional courtesy that helps your instructors improve their teaching approaches and course material; the majority of your instructors truly care about receiving your constructive feedback. Take a screenshot at the end of your submission confirming you completed the survey and upload it to canvas to earn the 5 points. Do *NOT* include any of your actual feedback/answers in the screenshot so as to ensure your submission remains anonymous.

Lab care and safety

Safety
A separate safety sheet will be available on canvas. Keep this handy at all times and strictly follow the rules and guidelines.

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 general 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.

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. Furthermore, to help promote safe practices, you will be required to do the following upon entering the room and before leaving:

At the beginning of your session:

- Wash your hands at the sink
- Wipe down your bench and tools with 70% EtOH

At the end of your session:

- Turn off gas
- Turn off under-shelf light
- Turn off water
- Lock microscope cabinet
- Wash your hands at the sink
- Wipe down your bench and tools with 70% EtOH. Be particularly thourough if there is oil, stain, or a bacterial spill on the bench.

COVID-restrictions
Since the beginning of the pandemic, we have all needed to be flexible with the rules and regulations regarding COVID precautions. Starting in the spring term 2022, masks will no longer be required to be worn indoors at the University of Oregon as per CDC guidelines (excepting health care settings on campus); however, individuals may still choose to wear them. Please be respectful of each individual's decision regarding masks. Furthermore, since policies are constantly in flux, please realize the University's stance on masks could change at any time. Last, while not required, you are encouraged to partake in regular COVID testing provided by the Monitoring and Assessment Program (MAP) at the University of Oregon. It is free and testing is conducted via collection of saliva. For more information on these services and to pre-registrater weekly, please use the following web address:

- https://coronavirus.uoregon.edu/map-testing

Buddy-system

You will be required to adhere to a buddy-system when you work in lab after hours (6:00pm-9:00pm M-F) and on weekends. Buddy-systems in scientific research labs are common and meant to help protect an individual's safety (not being alone in buildings and to protect against lab-related issues like bacteria/fire/chemicals/gasses/etc). You will need to coordinate coming into lab with one of your fellow Bi331 students (either from your direct lab section or from one of the other lab sections). You are not to allow non-enrolled friends (or anyone else) into the building. Any access point in the Science Complex allows people to roam freely though too many spaces and buildings, and there have been some issues in the past with people holding the doors for others. Not adhering to these rules could lead to the loss of building/Klamath 33 key card access at any time during the quarter. To set up your key card access, please work with Annie Rogers in the Biology office (*arogers2@uoregon.edu*); she will require your University identification number (9 digits long) and the alphanumeric prox number (4-5 digits), both of which are found on the backside of your student ID card. If any issues arise relating to your key card access over the term, please contact her directly.

Slack

To help you coordinate with your fellow students regarding experiments, coming to lab after hours and on weekends, and/or meeting to study, a class Slack account will be made. Please make sure to download the app on either your phone and/or your computer; it functions similarly to other texting apps but without you having to share your cell phone number. You will need your uoregon email to join our class Slack channel. Please remember that this channel is an extension of the University, and all codes of conduct will apply. If evidence of inappropriate behavior or cheating is detected, you risk being ejected from the Slack channel, along with any other appropriate disciplinary measures.

The Slack channel is designed to be used solely by you and your classmates and not to communicate with the teaching staff. All questions should be sent to your instructor and GEs via email and all official class communication will be done through canvas announcements.

Cleaning glassware

Reuseable glassware (test tubes, flasks, beakers) must be rinsed in the sink and then placed in the large wash tub. Remove tape and any other labeling. Graduated pipettes (1 ml, 5 ml, 10 ml) must be placed in the pipette cylinder next to the sink. All spectrophotometer and durham tubes should be bleached and tossed in glass waste boxes.

Sterile supplies

Sterile liquids (water, saline, mineral oil, etc.) are single use items; that is, if you take some portion from a tube or vial, do not return the tube or vial to the rack or shelf from which you took it. Sterility cannot be guanarateed for the next user. Unused portions are to be discarded. Agar plates removed from their bags, but that have not been opened, may be returned to the plate tub at the center island bench.

Stain bottles

You will use a lot of stains throughout the term. When a stain bottle runs out, place it in the specified container on the wash cart and retrieve a full bottle from the stains kept in cartons on the shelf on the east wall.

Microscopes

You will be assigned a microscope that you will use throughout the term. Three other students (one each from the other three 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 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 over $20!). 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 in the past.

Each supply drawer should have a small dropper bottle of immersion oil for microscopy. These bottles invariably leak, so always keep the oil bottle in the small container in which it is found in your tool tray.

Labeling

You will use dozens of tubes, flasks, and Petri dishes throughout the term, and keeping track of your cultures and enrichments will be crucial. Use fine point Sharpies and write DIRECTLY ON THE GLASS for the labeling of all serial dilution tubes, spectrophotometer tubes, flasks, bottles, and differential test tubes. Do not label plastic or metal caps of tubes or flasks (we can use tape for this if necessary), and never label the frosted glass area on a flask or tube. Always include your initials and lab section (I, II, III, or IV) and whenever appropriate, the source of material.

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 stored in the inverted position with the agar side up. This prevents condensation that forms in the lid from dripping onto the colonies on the agar (which can cause contamination or cells to spread into films rather than remain in isolated colonies).