

BIOL3314 General Micro Lab

Spring 2022 | M 2:00 – 4:50pm | Bolin 223

Instructor Information

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Office Hours: TWR 11:00am-1:00pm

General Information

Description

This course is an introduction to the biology of microorganisms including viruses, bacteria, archaea, protozoa, and fungi. Cell structure and function, metabolism, information flow and genetics, evolutionary relationships, and microbial ecology will be covered. A conceptual and experimental background sufficient to enable students to successfully pursue more advanced courses in related fields is provided.

Required Materials

1. **ACCESS TO D2L:** Laboratory papers and protocols as well as other materials will be posted on D2L, so you need access to it. Any issues with accessing D2L if you are enrolled in the course should be addressed to the IT department.
2. **DISPOSABLE LAB COAT** (or an actual lab coat). All lab coats **MUST** remain in the lab during the semester; you may **NOT** use the same lab coat for more than one class.
3. **SAFETY GLASSES OR GOGGLES.** All safety glasses or goggles **MUST** remain in the lab during the semester; you may **NOT** use the same glasses/goggles for more than one class.
4. **DISPOSABLE FACE MASKS will be provided.** You are **REQUIRED** to wear the mask at all times in the laboratory and the masks must not leave the lab. You may **NOT** opt out of this.
5. **LABORATORY NOTEBOOK.** You will need a **composition notebook** to use as a lab notebook. It is to remain in the lab at all times unless you have asked for and received permission to remove it.
6. **GLOVES will be provided.** However, if you require specialty gloves due to allergies, we ask that you provide those on your own. Gloves must be worn at all times in the lab.

Laboratory Attendance Policies

1. Laboratory attendance is required. Students who miss **THREE (3)** or more laboratory sessions, **for any reason**, will fail the lab, no exceptions.
2. Students are expected to always be **ON TIME**, to have read all required materials before walking into class, and to be willing to learn.
3. Students are expected to be **absolutely** prepared to do the day's laboratory work.

Late Assignment Policy

No late assignments will be accepted from any student for any reason. This policy is not negotiable. All class assignments are due **IN LAB AT THE BEGINNING OF LAB** on the dates specified.

Missed Quiz Policy

There will be no make-ups for missed quizzes. This policy is not negotiable. Quizzes must be done in lab at the beginning of lab.

Laboratory Grade

The laboratory counts as 40% of your overall grade in BIOL3314. The lab grade is broken down as follows:

Lab Quizzes (11 at 15 points each; lowest dropped)	40%
Mini Report: Soil Dilutions	10%
Mini Report: Fungi	10%
Major Report: Community Sampling of Water	20%
Completed Lab Notebook (Due at end of semester)	10%
Includes technique assessments	
Final Exam (Written; taken in lab)	10%

Laboratory Safety¹

All laboratory safety guidelines must be followed AT ALL TIMES. Failure to follow these basic safety rules—and others that will be told to you specifically throughout the semester—will result in your dismissal from the course.

1. **Absolutely NO FOOD OR DRINK is allowed in the lab for any reason or at any time.** This includes gum or candy. Nothing is to go into your mouth in lab. All water bottles, etc. must either remain outside of the room, or they must be completely zipped up inside your backpack. They may not be in the side pockets of your backpack.
2. **Lab coats must be worn at all times.** This lab coat must be stored in the lab when not in use. You may NOT use one lab coat for multiple classes.
3. **Appropriate clothing must be worn at all times.** This includes CLOSED-TOE SHOES and long pants, NOT yoga pants, tights, jeggings, or any other tight fitting pants. Note: Crocs are NOT shoes. Skimpy clothing and high-heeled shoes are NOT appropriate lab attire. Anyone wearing inappropriate clothing or shoes will be dismissed from the lab and will not be allowed back into the lab until proper attire is being worn. If that means you miss lab, you receive a zero for the day.
4. **No fake nails and all hair must be worn off the face and behind your head so you can see clearly and hair does not fall into your work.** Fake nails present a hazard in the lab and every time you use a gloved hand to push your hair out of the way you potentially contaminate yourself.
5. **Safety glasses or goggles must be worn at all times in the laboratory.** These glasses or goggles must be stored in the lab when not in use. The only time glasses or goggles may be removed is when actually looking through the lenses of a microscope.
6. **A face mask must be worn at all times in the laboratory.** This is to prevent inhalation or exhalation of potentially disease-causing microbes. You may not under any circumstances opt out of this. No, you don't need to take off a mask to use a microscope—the mask stays on!!
7. Students must maintain good laboratory practices. **Treat all microorganisms as potential pathogens.** While most microorganisms are not pathogenic to humans and have never been shown to cause illness, under unusual circumstances a few microorganisms that are not normally pathogenic can act as pathogens. Treat all microorganisms—especially unknown cultures—as if they were pathogenic. A student who has a compromised immune system or has had a recent extended illness should talk with the instructor before working in the microbiology laboratory.
8. **Treat all materials as cleanly as possible to avoid contamination.** Understand the operation and safe use of all equipment and materials needed for the laboratory prior to using them so as to avoid accidents and injury. Other people use the lab so make sure to clean up after yourselves.
9. **Disinfect work areas before and after use.** Use disinfectants provided in the laboratory to keep your area clean and safe to use. Never assume that someone else has cleaned the bench before you got to lab; always clean it yourself before and after class.

¹ Partially adapted from Carolina Biological Supply safety guidelines.

10. **Keep all personal items under the benches along the walls of the room.** Do not keep possessions on the bench tops or next to your feet in the aisles as this creates a safety hazard. Any personal items that become contaminated will be autoclaved and disposed of, regardless of what it is or how much it cost you. **THIS INCLUDES YOUR PHONES! Note: using your phone with gloved hands just ensures you contaminate yourself after class.**
11. **Wash your hands.** Use a disinfectant soap (i.e., the soap in the lab) to wash your hands before and after working with microorganisms.
12. **Never pipette by mouth.**
13. **Label everything clearly.** All cultures, chemicals, disinfectant, and media should be clearly and securely labeled with their names and dates. If they are hazardous, label them with proper warning and hazardous information. Unlabeled materials will be thrown out.
14. **Autoclave or disinfect all waste material.** All items to be discarded after a class, such as culture tubes, culture plates, swabs, toothpicks, wipes, disposable transfer needles, and gloves, should be placed in the biohazard autoclave bag provided. Paper towels, Kleenex, paper, packaging, and such should be thrown out in the regular trash cans, not the biohazard bins. **ONLY** glass goes in the glass waste (not paper towels or anything else).
15. **Clean up spills with care.** Cover any spills or broken culture tubes with a 70% ethanol or 10% bleach solution; then cover with paper towels. After allowing the spill to sit with the disinfectant for a short time, carefully clean up and place the materials in a biohazard autoclave bag to be autoclaved. Wash the area again with disinfectant. **Never** pick up glass fragments with your fingers or stick your fingers into the culture itself; instead, use a brush and dustpan. If working with animal or plant pathogens, keep the area clear and notify your instructor.
16. **Laboratory notebooks and the pens used to write in them MUST remain in the lab.** Paper picks up microorganisms from the environment and we do not know exactly what sorts of organisms we will be working with in the lab; thus, for safety, all notebooks and pens must remain in the lab.

Laboratory Notebooks

Many of the labs we will be doing are coming from papers and other non-traditional pre-formatted lab protocols (i.e., they are being developed as you work, much as they would in a real research lab). As such, you will be required to write your OWN protocols from the papers you read and make flow-charts to help you move through the lab protocols smoothly. To help you learn how to do this, you will keep a laboratory notebook.

How to Keep a Notebook:

1. Leave the first page blank for inserting a table of contents. Corollary: this means you need to NUMBER the pages of your composition book.
2. **Write ONLY on the right-hand page.** Leave the left-hand page blank so you can tape in flow charts, pictures, etc. and make notes/observations on what you do as you do it.
3. Include the DATE each time you come to the lab to do something.
4. For each specific experiment, include the main idea of what you are doing and why. This should just be 1-2 sentences prior to writing down protocols/what you are doing/did in the lab.
5. **Everything you do must be written into the lab notebook.** Even though you are following a protocol, you do not always do what is written in the protocol; sometimes things get changed as you do them, you have accidents, you make observations (or you SHOULD), and so forth. All of this must be written down such that someone can come along behind you and repeat what you did. You never know when one little change will make the difference between a successful and unsuccessful experiment. **YOU NEED TO MAKE NOTES AS YOU DO THINGS.** You will NEVER remember things at the end of the semester—write up the day every day; don't put it off.
6. **Write down all of your results and your observations.** For example: if you are counting colonies you need to report the colony count, but also exactly how long was the plate incubated, what the colonies look

like, are they all the same, are they different, etc. Pictures should be annotated as to what they are. You should always write up a short discussion of your data after you collect it so that you can remember later what it was you actually observed and what it means. If you don't write things up as you collect them, you will forget and it will be IMPOSSIBLE to write a good lab report.

7. Any calculations you do or data you look up from outside sources (such as a known sequence for a particular bacterium or phage, for example) must also be included in your notebook with references, if applicable.
8. Once a given protocol is "completed", you should write up an overall conclusion for what was done and what was found. This is especially important for investigative type experiments. Discuss results obtained with respect to anticipated findings, any errors that may have caused your results to deviate from what was expected, and any other information that helps explain what you obtained. **Summarize what you learned. Discuss your data!**
9. **Write what you do when you do it.** Periodically the notebooks will be checked to ensure you are actually writing in them as you go and NOT attempting to write everything at the end for a grade. At the end of the semester when I grade the notebooks, I will randomly open it and read what I find—if I can't immediately tell what you were doing and why from that snippet, then you will NOT get a good grade on your notebook.

Lab Report Guidelines

All lab reports should include the following. You are to adhere to these guidelines—I do NOT care how you write reports for other classes, nor do I care how YOU want to write the report. You will write it as stated here.

1. A **descriptive title** (if the words "Lab Report" appear anywhere in your report, you will receive a zero for the entire manuscript). A descriptive title actually describes the overall result of the work—it is not a question, it is not so generic I won't know what the report is about, nor is it so wordy as to be ridiculous. Look at REAL papers to see how titles are written!
2. A **SHORT introduction** that says basically what was done and **why we did it** (actual work in context); the introduction does NOT say what you found and NEVER uses the phrase "the purpose of this experiment was" (using this statement will also net you a zero for the entire manuscript). READ actual papers to see how things are set up. Usually, basic background is given that leads to a question, that leads to a hypothesis that was tested and now is being written up.
3. A **methods** section written in paragraph form that reflects what you ACTUALLY did in the lab—this is part of the reason for keeping a lab notebook! Read some papers to get a feel for the methods section and use them as models. Methods sections are short and to the point. **No one cares that you labeled your tubes**, but we do care **what was in the tube**. Again, READ papers to see how the methods are written. Never begin sentences with actual numerals and put spaces between numerals and their units. Methods contain only what is needed for someone to repeat what you did.
4. A **results** section composed of text and figures/tables. A results section is NOT simply a bunch of poorly labeled figures or tables with no explanation of what the reader is looking at. **A results section ALWAYS begins with text that introduces an experiment, refers to a figure or table, and then presents that figure or table, followed by a short summary statement of what I should "see" when I look at that figure or table.** You must walk the reader through the results you obtained, whatever those results are. You do not comment on the results, you simply present them. READ SOME REAL PAPERS!!! There is no excuse for not being science literate. Note: the first thing I check in a report is whether the results section begins with text and whether the figures and tables are prepared correctly. If the results section is not prepared correctly, I don't even bother reading the rest because the report has already failed.
5. A **discussion** section where you interpret your results, discuss errors or problems, and make suggestions for future work. **NEVER** blame strange or poor results on "experimenter error"! Unless you KNOW you did something wrong, you must provide biological explanations for your results and support your conclusions with the data obtained!
6. A **reference** section for ANY work you pull into the report that is not yours or your groups.

All lab reports are to be written in past tense, third person, passive voice. You **NEVER** use “I” or “we” and the experiments are already done, so you always write in past tense. You must use complete sentences and proper grammar as these will form part of your grade. You must proofread your reports prior to turning them in; correct your errors and make sure your report is a cohesive, coherent manuscript. You may discuss labs with other members of your group, but all aspects of the lab report are to be individually generated unless it is a photograph (photographs that are shared within a group must be given proper citation as to whose photo it is).

Lab reports should be typed in their entirety (do NOT make corrections or additions/deletions after printing in pen or pencil; you need to proof before you print!). Reports should be in 12pt Times New Roman ONLY; 1.5 spacing for text, single spacing for tables, titles, captions; 1 inch margins all the way around; page numbers in top right corner.

Failure to follow these directions for presentation of a lab report will result in failing grades. **I am not kidding.** In most cases, if something is improperly formatted, I won't even read it and will simply give it a zero.

(Tentative) Lab Schedule

IMPORTANT NOTE: STUDENTS MAY BE EXPECTED TO COME IN TO LAB OUTSIDE OF SCHEDULED LAB HOURS.

DATE	LAB PROJECT	WHAT IS DUE
Jan 10	Lab will not meet this first week.	All lab materials (lab coat, goggles, notebook) due on Jan 24. No materials = ZERO for the day.
Jan 17	MLK – NO LAB	
Jan 24	Lab Introduction; student check-in; safety lecture General Microscopy (no oil) of prepared slides Demonstrate knowledge of microscope	READ your syllabus before class REVIEW microscope usage on D2L Signed safety sheet Signed check-in sheet You must show me a focused specimen under the microscope
Jan 31	Aseptic Technique and Oil Immersion Microscopy Aseptic Technique w/o organism Aseptic Technique w/ <i>Staphylococcus epidermidis</i> Smear and Simple Stain of <i>Staphylococcus epidermidis</i>	READ general info on D2L QUIZ #1
Feb 7	Dilutions and Plate Counts* Serial Dilutions of Soil Samples → plate dilutions Streak Plate Practice (Isolation) Streak Plate of Mixed Culture (practice isolation) ANALYZE Aseptic Technique	READ general info on D2L QUIZ #2 You must show me your aseptic technique results
Feb 14	Community Sampling of Water Samples* Day 1 Prepare EcoPlates → Begin incubation Dilutions and Plate Counts READ Plate Counts COLLECT Class Data Streak Plate Practice ANALYZE streak plate technique Streak new plates (2 nd plate)	READ general info on D2L QUIZ #3 You must show me your streak plates
Feb 21	Community Sampling of Water Samples Day 2 ANALYZE community data Streak from lactose well of EcoPlate to EMB and TSA (each student in group will isolate <i>E. coli</i>) Streak Plate Practice ANALYZE 2 nd plates DISCUSS Mini Report #1 (Soil Dilutions)	QUIZ #4 You must have a rough draft of Mini Report #1
Feb 28	Community Sampling of Water Samples Day 3 Streak from EMB → TSA (isolation) ANALYZE TSA plates (count diversity)	MINI REPORT #1 DUE QUIZ #5
Mar 7	Community Sampling of Water Samples Day 4 Prepare Biolog plates to confirm <i>E. coli</i> isolate Begin overnight cultures of <i>E. coli</i> isolate Gram stain <i>E. coli</i> isolate	READ general info on D2L QUIZ #6 You must show me your Gram stain
Mar 14	SPRING BREAK – NO LAB	
Mar 21	Community Sampling of Water Samples Day 5 ANALYZE Biolog plates Kirby Bauer Antibiotic Susceptibility Testing Prepare plates with discs → Incubate	READ general info on D2L QUIZ #7 You must show me your Kirby Bauer plates
Mar 28	Community Sampling of Water Samples Day 6 READ Kirby Bauer Plates COLLECT class data Environmental Sampling of Fungi* Top half of phone → swab and plate on SAB Bottom half of phone → swab and plate on Mycosel agar	READ general info on D2L QUIZ #8

DATE	LAB PROJECT	WHAT IS DUE
Apr 4	Environmental Sampling of Fungi Germ Tube Assay for <i>Candida albicans</i> COLLECT class data DISCUSS Community Sampling Reports DISCUSS fungi report	READ general info on D2L QUIZ #9
Apr 11	Hunting Tardigrades Finding water bears in various samples → dissecting scopes Examining water bears using light microscopy	MINI REPORT #2 DUE READ general info on D2L QUIZ #10 Tardigrades, if found, must be shown to the class
Apr 18	Hunting Protozoa Protozoa from water samples (wet mounts with protoslo) Protozoa from soil samples (wet mounts with protoslo) Laboratory Check Out	MAJOR REPORT DUE QUIZ #11 Signed check out sheet
Apr 25	Laboratory Final	

*=will be written up as a report.