BIOL3314-201 Microbial Life Lab

Spring 2019 | F 2:00 – 4:50pm | Bolin 223

Instructor Information

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Office Hours: MWF 9:00-11:00; MW 12:00-2:00pm

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. **LABORATORY NOTEBOOK.** You will need a composition notebook to use as a lab notebook. It is to remain in the lab at all times.

Suggested Websites

The following websites will prove useful: [American Society for Microbiology](https://www.asm.org/); [Phages Data Base](https://phagesdb.org/)

## Laboratory Attendance Policies

1. Laboratory attendance is required. Students who miss THREE (3) or more laboratory sessions, **for any reason**, will fail the lab.
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.

## Laboratory Safety[[1]](#footnote-1)

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.
3. **Appropriate clothing must be worn at all times.** This includes CLOSED-TOE SHOES and clothing that at least reaches the knee. 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.
4. **Safety glasses or goggles must be worn at all times in the laboratory.** These glasses or goggles must be store in the lab when not in use.
5. 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.
6. **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.
7. **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.
8. **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.
9. **Wash your hands.** Use a disinfectant soap to wash your hands before and after working with microorganisms. Nondisinfectant soap will remove surface bacteria and can be used if disinfectant soap is not available. Gloves may be worn as extra protection.
10. **Never pipette by mouth.**
11. **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.
12. **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.
13. **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.
14. **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. 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.
2. Write ONLY on the right hand page. Leave the left hand page blank so you can tape in flow charts, pictures, etc.
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; somethings 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.
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.
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.

(Tentative) Lab Schedule

**IMPORTANT NOTE: ALL STUDENTS WILL BE EXPECTED TO COME IN TO LAB OUTSIDE OF SCHEDULED LAB HOURS AND TO OCCASSIONALLY GO OUT INTO THE FIELD TO COLLECT SAMPLES. IF YOU ARE UNWILLING TO COME IN OUTSIDE OF SCHEDULED LAB TIMES, DO NOT TAKE THIS COURSE.**

| **DATE** | **LAB PROJECT** | **WHAT IS DUE** |
| --- | --- | --- |
| Jan 18 | Lab Introduction***Prior to next lab: purchase lab coat, goggles, and composition book for lab.*** | Nothing |
| Jan 25 | **Microbial Challenge 1:** Characterization of Clinical Isolates1. Isolate colonies from mixed clinical sample
2. Use the compound microscope to view wet mounts
 | Quiz #1 |
| Feb 1 | **Microbial Challenge 1:** Characterization of Clinical Isolates1. Streak plates for isolation
2. Gram Staining
 | Quiz #2 |
| Feb 8 | **Microbial Challenge 1:** Characterization of Clinical Isolates1. Metabolic characterization of isolates
2. Identification of unknowns

***Prior to next lab: Collection of water and soil samples*** | Quiz #3 |
| Feb 15 | **Microbial Challenge 2:** Bacteriological Examination of Water1. Filtration of water samples

**Microbial Challenge 3:** Phage Hunting1. Screening of soil samples
 | Quiz #4 |
| Feb 22 | **Microbial Challenge 2:** Bacteriological Examination of Water1. Analyze plates
2. Pick colonies and streak for isolation
3. Gram staining

**Microbial Challenge 3:** Phage Hunting1. Identify Putative Plaques
 | Quiz #5**Lab Report on Clinical Isolates is DUE** |
| Mar1 | **Microbial Challenge 2:** Bacteriological Examination of Water1. Re-streak for isolation
2. Metabolic testing of pure cultures
3. Frozen stocks

**Microbial Challenge 3:** Phage Hunting1. Plaque Purification
2. Spot-test to see if isolated phages can kill coliforms from water lab
 | Quiz #6 |
| Mar 8 | **Microbial Challenge 2:** Bacteriological Examination of Water1. Kirby Bauer testing for antibiotic resistance

**Microbial Challenge 3:** Phage Hunting1. DNA Extraction
 | Quiz #7 |
| Mar 15 | **Microbial Challenge 3:** Phage Hunting1. Restriction digests of phage
2. Gel analysis of restriction digests
 | Quiz #8 |
| **Mar 22** | **SPRING BREAK NO CLASS** |  |
| Mar 29 | **Microbial Challenge 3:** Phage Hunting1. RAPD PCR Analysis of Phages
 | Quiz #9**Lab Report for Water Analysis is DUE** |
| Apr 5 | **Microbial Challenge 3:** Phage Hunting1. Run agarose gels of PCR reactions
2. Using restriction digests and RAPD for phylogenies

**Microbial Challenge 4:** Isolation of *Candida* sp.1. Swab oral cavity and plate
 | Quiz #10 |
| Apr 12 | **Microbial Challenge 4:** Isolation of *Candida* sp.1. Streak for isolation (CHROM agar)

***Prior to next lab: collect moss or lichen and a water sample with sediment from Sikes Lake*** | Quiz #11 |
| **Apr 19** | **HOLIDAY BREAK NO CLASS** |  |
| Apr 26 | **Microbial Challenge 5:** Tardigrades and Protozoa1. Examination of moss for tardigrades and characterization of tardigrades
2. Filtration of water sample for protozoa
3. Microscopic analysis and identification of protozoa
 | Quiz #12**Lab Report for Phage Analysis is DUE** |
| Apr 3 | Final Exam (Lab Final) | **Lab Report on Isolation of *Candida* sp. (with class data) is DUE** |

## Lab Report Guidelines

All lab reports should include the following:

1. A **descriptive title** (if the words “Lab Report” appear anywhere in your report, you will receive a zero for the entire manuscript).
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).
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.
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.
5. A **discussion** section where you interpret your results, discuss errors or problems, and make suggestions for future work.
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.

## Laboratory Grade

The laboratory counts as 30% of your overall grade in BIOL3314. The lab grade is broken down as follows:

 Lab Quizzes (12 at 15 points each; lowest dropped) 40%

 Microbial Challenge 1: Clinical Isolate Report 10%

Microbial Challenge 2: Water Analysis Report 10%

 Microbial Challenge 3: Phage Report 10%

 Microbial Challenge 4: *Candida* sp. Report 10%

Completed Lab Notebook 10%

 Final Exam (Written) 10%

1. Partially adapted from Carolina Biological Supply safety guidelines. [↑](#footnote-ref-1)