

Course Syllabus: General Microbiology Laboratory

General Information:

Course Meetings:	M (2:00 – 4:50 pm OR 5:30 – 8:20 pm) Bolin Hall 223
Instructor:	James Masuoka, Ph.D.
Office:	Bolin Hall 324
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Office Hours:	MWF 10:00 – 10:50 am; TR 9:00 – 10:50 am (Other times by appointment)

Required Texts:

There is no required lab manual for BIOL 3314 (Sp23). Laboratory exercise theory and procedures will be provided through D2L.

Lab coat (gloves, safety glasses, and mask will be provided)

Course Description (Laboratory):

This laboratory section complements the lecture portion of the course as a study of prokaryotic structure and physiology. Students will learn and apply practical laboratory techniques to the identification and characterization of bacteria.

NOTE: Bacteriology relies on studying living organisms. As a result, time is required for organisms to grow after inoculation into growth medium. Thus, students will be required at times to come into the lab outside of the scheduled laboratory period to collect data and interpret results.

Course objectives (Laboratory):

- Practice general laboratory safety
- Practice safe handling of microorganisms, including aseptic technique
- Recognize various cellular and colony morphologies of prokaryotes
- Correctly use and maintain a compound microscope
- Use pure culture and selective techniques to enrich for and isolate microorganisms
- Properly prepare and view microbial specimens for examination using microscopy (bright field)
- Interpret and analyze results from various assays used to identify bacteria and apply these methods unknown isolates
- Correctly document laboratory work in a formal laboratory notebook
- Communicate experimental results and analysis in a written format

General Laboratory Policies:

- Laboratory safety rules **must** be followed at all times (see following section). Safety violations will result in deductions from the laboratory participation grade (see below).
- Cell phones and pagers are to be turned off before lab begins.
- Students are expected to read the introductory material prior to each lab session.
- Cheating will not be tolerated. Anyone suspected of cheating will be subject to the consequences outlined in the University's academic honesty policy.
- All lab sessions must be attended. We will not offer make-up labs.
- Punctuality is expected at all times. If you are more than 10 minutes late, you will miss the quiz for the day and be counted as absent.
- Attendance in lab is required. Attendance policy regarding excused absences is provided above.
- All quizzes, exams, or assignments missed due to unexcused absences will be recorded as zeros.
- If you have a documented disability that will impact your work in this class, please contact the TA or instructor to discuss your needs.
- Each group is responsible for proper clean up at the end of the laboratory period. This includes proper cleaning and storage of microscopes, proper disposal of contaminated materials, disinfection of benches and workspaces, etc. (see Waste Disposal Guidelines & Clean-up below)
- **Three unexcused lab absences will result in failure (receiving 0 points) of the laboratory portion of the course.**

Attendance Policy:

General and specific policies regarding attendance in BIOL 3044 Laboratory sections are provided in the sections above.

Grading:

Quizzes:	100 points (10 of 11 quizzes)
Assignments:	100 points (5 x 20 points)
Participation/safety:	70 points
Research Forum evaluation	30 points
Laboratory Notebook	40 points
Unknown isolate research report	60 points
Total:	400 points

Lab constitutes approximately 40% of the BIOL 3314 grade.

Quizzes will be given at the 5 minute mark of the laboratory period. Thus, it is imperative that you arrive to lab on time and are ready to go at the start (see General Course Policies). Quizzes will cover the exercises from the previous week and material from the upcoming exercises. Thus, it is important both to understand your previous

results and to have read the exercises for the week so that you are prepared. Over the course of the semester, 11 quizzes (10 points each) will be given. The lowest score will be dropped.

There will be **4 assignments** that will evaluate **key laboratory skills**. Each assignment will be worth 20 points (80 points total). The first will focus on correct use and care of the compound microscope. One will focus on using correct aseptic technique – being able to transfer cultures without contaminating the culture or the environment. One will focus on the streak plate technique of isolating colonies. One additional, **in-lab assignment** will focus on microscopy and observations (20 points).

The major project for this semester will be isolation and identification of one-two bacterial isolates from the soil by culture-based and DNA sequence methods. The target phenotype will be production of antimicrobial agents. Isolates will be characterized using metabolic assays. In addition, genomic DNA will be extracted from each of the isolates and the 16S rDNA will be amplified by PCR and submitted for DNA sequencing. The data collected during enrichment, isolation and identification will form the basis of a **final research report** (60 points). A more detailed description of the project will be handed out later in the semester.

Additional isolations will include: filamentous fungi from soil; yeasts from cheek swabs; bacteriophage viruses from a wastewater sample; and protozoa from local water sources.

All data, results, observations, and other experimental notes will be recorded in a **laboratory notebook**. The notebook will also be used to record observations and results from all laboratory exercises throughout the semester. Guidelines for proper laboratory notebook keeping will be provided in the lab and on the lab D2L webpage. All notebooks will be evaluated throughout the semester, with the final assessment at the end of the semester (40 points).

The **laboratory participation/safety** grade is based on adherence to laboratory safety and attendance policies. Everyone starts the semester with 70 points. During the first two lab periods, students will be given gentle reminders regarding lab safety and attendance as needed. After that, points will be deducted for each violation. The severity of the deduction is at the discretion of the instructor. The exception to this regards appropriate dress. As stated above, students wearing open-toed shoes or short pants/skirts will not be permitted to enter the laboratory.

Each semester, the University sponsors the **Undergraduate Research and Creative Activity Forum**. In this Forum, students present their research findings or creative works. For this assignment, students will **critically evaluate** three (3) poster presentations or three (3) oral presentations (or combination thereof). This will provide experience in both how to present results and how to critically evaluate data present by others in preparation for the Case Study reports at the end of the semester. Satisfactory completion of this assignment will be awarded 30 points towards your laboratory/course grade.

Tentative Schedule of Laboratory Exercises

Week	Date	Exercise*	Quiz	Assignment
1	Jan 16	No Lab – MLK Jr. Day		
2	Jan 23	Intro; Lab Safety; Lab Notebooks; Microscope; Pipets Field paperwork		Handout: 1, 2, 3, 4
3	Jan 30	Aseptic Technique; Protozoa (wet mounts) Collect Soil	1	5 (in lab)
4	Feb 6	Soil prep: sieve, dilutions, plate (for bacteria) Streak plate	2	
5	Feb 13	Pick colony; subculture Preparing bacterial smears; Gram Stain	3	
6	Feb 20	Subculture (2); Smear & Gram Stain Growth in broth (frozen) & on slant	4	Pure culture
7	Feb 27	Prepare frozen stocks; Test for antibiotic production	5	Pure culture
8	Mar 6	Subculture (for over break); Work on assignments	6	
9	Mar 13	No Lab – Spring Break		
10	Mar 20	Subculture (BUG, broth) Soil prep: dilutions, plate (for fungi)		
11	Mar 27	Set up BIOLOG plates, read (24/48 hr) Prep bacterial DNA, run PCR; Check fungal plates	7	
12	Apr 3	Agarose gel of PCR product, send for sequencing Cheek swab: plate; Tape prep	8	
13	Apr 10	Germ tube assay; Subculture to ChromAgar Tape prep of moulds, wet mount of yeasts	9	
14	Apr 17	T4 phage titer: dilution, plaque assay	10	
				Forum (4/20)
15	Apr 24	Wastewater: dilution, plaque assay (<i>S. aureus</i> phage) Collect pond water sample	11	Reports due
16	May 1	Protozoa wet mounts (collected water); Check out		

* Note on experimental cultures: Growth or other results should be read after 24 – 48 hours (although some slow growers may require 72 hours). So, ideally, you will arrange to come into lab outside of your scheduled time to observe your results. If this becomes problematic, after the required growth period the cultures can be transferred to the refrigerator (4°C) until the following Monday. Keep in mind, however, that even with the lower temperature, the organisms will continue to metabolize and your results may change.

Laboratory Safety:

Microbes are found everywhere within the environment – in the air, on surfaces, on your body. In the Bacteriology Laboratory, we deal with microbes at higher concentrations than found in the environment. We will also be dealing with organisms that are potentially pathogenic to humans – thus we treat EVERY organism as if it were pathogenic.

1. No food or drinks are to be taken into or consumed in the laboratory. Further, ANY activity that involves hand-to-face contact (applying cosmetics, handling contact lenses, etc.) should be avoided.
2. Disinfect the work area before starting lab, after completing lab, and after any spills that occur. Do not assume that the lab members before you cleaned up after themselves.
3. Wash your hands thoroughly with soap and water before leaving the laboratory – even if you need to leave only for a short time.
4. Open-toed shoes, sandals or similar footwear are not appropriate and should not be worn in the laboratory. Shorts and short skirts are also inappropriate in terms of laboratory safety. These regulations are for your personal safety. Students wearing inappropriate dress will not be permitted to enter the laboratory.
5. Long hair must be tied back as it is not only a potential source of contamination, but also a fire hazard.
6. Proper personal protective equipment (PPE) must be used in the lab whenever work is being done. For this laboratory, PPE includes a labcoat, safety glasses and laboratory gloves.
7. Be aware of the location of safety equipment such as fire extinguishers, eyewashes, showers, First Aid kits, etc.
8. Follow all waste disposal guidelines. (see below)
9. Refer to the Introduction section of your lab manual for additional discussion of laboratory safety issues.
10. When in doubt, ASK!

Waste Disposal and Cleanup:

Proper cleanup of the laboratory is essential to reduce contamination and to ensure that subsequent lab sections have a clean and organized work area. The following guidelines must be observed during each lab session. Each student must take an active role in proper cleanup and waste disposal. Do not leave it for someone else.

Lab benches: There are wash bottles of disinfectant (Cidecon) located on the lab benches. You must clean the lab bench before AND after each lab. If you should run out of disinfectant, refill the bottle from the large carboy next to the microscope cabinet. The best technique for disinfection is to stream disinfectant over the surface, then use a paper towel to even the fluid over the surface so that a light film remains. Do not wipe to dryness, but allow the disinfectant to air dry. The extended contact of the fluid to the surface increases effectiveness.

Paper towels and **soap** are located next to the sink.

Spills: Immediately cover any spilled culture material with paper towels to contain the spill and prevent it from spreading. Saturate the paper towels with disinfectant (Cidecon) and allow it to stand 15 minutes. Report the spill to the instructor. After the reaction time is done, remove the towels and dispose of them in the regular trash (all living cells should have been killed during the time exposed to disinfectant).

Waste material: Bacterial cultures must be killed prior to disposal. Each group must dispose of their cultures once they have obtained and recorded their results. Dispose of each type of waste according to the following guidelines.

- **Liquid cultures:** add bleach to the tube. Squirt bottles containing bleach (1:2 dilution of household bleach in water, 2.5% final) are kept next to the sink. Add 1/5 the culture volume (usually about 1 cm) and place the tube into the holding racks next to the sink. The tube cap should go into the appropriately marked basket. **NEVER** pour your cultures down the drain or into the trash.
- **Solid cultures (plates):** All cultures on plastic Petri plates are disposed of in the Contaminated Material Container (CMC, large box with the red plastic bag) next to the sink.
- **Solid cultures (slants):** For cultures on agar slants – remove the cap and place the cap in the appropriate basket. The tube is disposed of in the CMC. **DO NOT** bleach your slants.
- **Semi-solid cultures:** Some growth media are termed semi-solid because they do not contain enough agar to completely solidify. These media are treated like agar slants.
- **Additional note on CMCs:** Only materials that are visibly contaminated are to be put into CMCs. Paper towels used for washing hands go into the trash, as do transfer pipette wrappers, sterile swab wrappers and the like. If the organisms are dead/killed – as with paper towels used to wipe up Cidecon from the benches – the material still goes into the trash.