

BIOL 2144: Fundamental Clinical Microbiology Laboratory

General Information:

Lab Meeting Times: TR (1:00 – 2:20 pm or 3:30 – 4:50 pm) Bolin Hall 223
Instructor: Kaitlin Raber
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Office/Tutoring Hours: TR 11:00am - 1:00pm

Course Description:

This laboratory section complements the lecture portion of the course as an introduction to medically important bacteria, fungi, helminths, protozoa, and viruses. Students will learn and apply practical laboratory techniques to the study and identification of microorganisms, particularly bacteria, which affect the health and lives of humans. **NOTE:** Microbiology relies on studying living organisms. As a result, time is required for organisms to grow after inoculation into growth medium. We have made every attempt to restrict student activity to the scheduled periods. However, students may still need to come into the lab outside of the scheduled laboratory period to collect data and interpret results.

Required Text:

Microbiology Laboratory Theory & Application: Brief Edition by M J Leboffe & BE Pierce. 3rd Edition. Morton Publishing Company, 2016. ISBN: 978-1-61731-477-3

Other Required Materials:

Composition book (Laboratory notebook): This does not have to be anything fancy, but it should have a tape/cloth binding (as opposed to being spiral-bound). Non-perforated pages are best. I like the pages with grids, but lines are perfectly acceptable. A 50 page composition book should be plenty. This will be used to record your observations and data throughout the semester.

Course objectives:

1. Practice general laboratory safety
2. Practice safe handling of microorganisms, including aseptic technique
3. Recognize various cellular and colony morphologies of microorganisms
4. Correctly use and maintain a compound microscope
5. Use differential and selective growth media to obtain pure cultures
6. Correctly perform and interpret Gram Stains and other staining techniques
7. Correctly interpret results of tests used in the clinical identification of bacteria
8. Use the above techniques to identify unknown isolates of bacteria in a simulated patient specimen

General Laboratory Policies

1. All general course policies, as provided in the lecture syllabus, are held for the laboratory portion of the course.
2. Laboratory safety rules **must** be always followed (see section below). Failure to comply with these regulations will result in dismissal from the lab session and deductions from the laboratory participation grade (see below).
3. Cell phones and pagers are to be turned off before lab begins.
4. Students are expected to read the introductory material prior to each lab session.
5. Cheating will not be tolerated. Anyone suspected of cheating will be subject to the consequences outlined in the University's academic honesty policy.
6. Analytical and critical thinking skills in both written and oral communication are part of the learning outcomes of this course. Therefore, all writing assignments and classroom discussion responses should be prepared by the student. Developing strong competencies in this area will prepare you for a competitive workplace. Because of this, AI-generated submissions are not permitted and will be treated as plagiarism (Adapted from Texas Tech University statement).
7. All lab sessions should be attended. We will not offer make-up labs.
8. Punctuality is always expected. If you are more than 10 minutes late, you will miss the quiz for the day and be counted as absent.
9. Absences may be excused only by prior arrangement with the laboratory or course instructor. Absences will be excused for:
 - a. **Death of an immediate family member.** Immediate family members include: a grandparent, parent, sibling, spouse, in-law, aunt, uncle, or child.
 - b. **Summons to appear in court or jury duty.** A copy of the summons is required.
 - c. **Call to military service.** A copy of your orders to report is required.
 - d. **University sponsored event.** Members of athletic teams, college bowl participants, etc. will be excused with proper notification.
 - e. **Debilitating illness or disability.** Illnesses will be addressed on an individual basis. If a student is affected by an illness that is not debilitating, (i.e. flu, virus infection) which may result in the student missing one or more consecutive class sessions, that student will be marked as **unexcused** for days missed **unless a doctor's note is provided.**

ROUTINE APPOINTMENTS, medical or otherwise, AND VACATION TRAVEL ARE NOT ACCEPTABLE Reasons for excused absences

10. All quizzes, exams, or assignments missed due to unexcused absences will be recorded as zeros.
11. 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.
12. If you have a documented disability that will impact your work in this class, please contact the TA or instructor to discuss your needs.
13. The professor considers this classroom to be a place where you will be treated with respect as a human being - regardless of gender, race, ethnicity, national origin, religious

affiliation, sexual orientation, political beliefs, age, or ability. Diversity of thought is appreciated and encouraged, provided you can agree to disagree. Guns or other weapons create a coercive environment that is neither safe nor conducive to learning. Therefore, weapons of any kind will not be permitted in my classroom. This includes guns, concealed or otherwise, regardless of licensure. Any student bringing a weapon to class or to lab will be immediately dropped from the course. It is the professor's expectation that ALL students consider the classroom a safe environment.

14. The instructor reserves the right to amend these rules as needed throughout the term.

Attendance Policy:

Students are expected to attend all meetings of the classes in which they are enrolled. Although in general students are graded on intellectual effort and performance rather than attendance, absences may lower the student's grade where class attendance and class participation are deemed essential by the faculty member. In those classes where attendance is considered as part of the grade, the instructor should inform students of the specifics in writing at the beginning of the semester in a syllabus or separate attendance policy statement.

An instructor who has an attendance policy must keep records daily. The instructor must give the student a verbal or written warning prior to being dropped from the class. Instructor's records will stand as evidence of absences. A student with excessive absences may be dropped from a course by the instructor. Any individual faculty member or college has the authority to establish an attendance policy, providing the policy is in accordance with the General University Policies. [MSU Student Handbook, p. 61]

If you feel ill (esp. with signs and symptoms of COVID-19): Stay home and isolate yourself. Inform your instructor immediately of your circumstances.

Three unexcused lab absences will result in failure (receiving 0 points) of the laboratory portion of the course.

Grading:

Quizzes:	100 points
Assignments:	80 points
Unknown/Case report:	40 points
Labster simulations:	80 points
Forum Evaluations:	30 points
Participation/safety:	70 points
Total:	400 points

Lab constitutes approximately 40% of the BIOL 2144 grade.

Quizzes will be given in the first 10 minutes of one laboratory period per week (see General Course Rules). Quizzes will cover the exercises from the previous lab and material from the upcoming exercises (including materials from the online pre-labs). Thus, it is important both to understand your previous results and to have read the exercises for the week so that you are prepared.

There will be **4 assignments** that will evaluate **key laboratory skills**. Each assignment will be worth 20 points. One will focus on the use of proper aseptic technique. One will focus on correct use and care of the compound microscope. One will focus on successful completion of a Gram stain. One will focus on the streak plate technique of isolating colonies.

The **patient sample unknown** will be a simulated sample based on an actual case study. Each student will be responsible for identifying one isolate from their group's sample. Identification of the isolates will be conducted using traditional culture and morphological assays, along with biochemical/metabolic tests. The results and conclusions of this analysis leading to the identification of the unknown organism will be written up as a brief **case study report**. This report will also put your unknown organism in the context of the case and present your analysis of the patient's case – background, diagnosis, treatment, etc. A separate project sheet describing expectations in more detail will be handed out around mid-semester. Throughout the semester, you will be recording your observations in a **laboratory notebook**. Your records from the notebook will greatly enhance your ability to write a good lab report as well as provide material you can use to prepare for lab quizzes.

This semester, **lab simulations through Labster** are included as the pre-lab preparation. Each selected simulation covers an important concept or provides experience with a particular technique. Each simulation has associated questions. Completion of the Labster simulations constitutes 20% (80 points) of the lab grade.

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.

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 week or two of lab, 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.

Tentative Schedule of Laboratory Exercises

Week	Date	Exercise	Topic
1T	Aug 29		Labs do not meet
1R	Aug 31		Labs do not meet in person Complete online lab safety videos – see D2L
2T	Sept 5	1-1; 1-2	Check-in, Laboratory Safety, Glo-Germ
2R	Sept 7	2-1	Notebook tutorial; Ubiquity
3T	Sept 12	1-4; 3-1	[Read results]; Aseptic Technique (A1) ; Microscope Use (A2)
3R	Sept 14	3-3; 1-5	[Read results]; Examine Eukaryotes (prepared & wet mount); Streak plate (from 2-1) (A3)
4T	Sept 19		[Read results]; Examine Bacteria (prepared & wet mount) (from streak plate)
4R	Sept 21	3-4; 3-6	Making smears; Gram stain (from streak plate) (A4)
5T	Sept 26		Koch's Postulates experiment: milk/yogurt (wet mount, streak plate)
5R	Sept 28		Inoculate milk (from streak plate) Gram stain (from streak plate); A2 due*
6T	Oct 3	4-1; 4-2; 4-4; 4-5	[Read results]; Streak plate ("infected" milk) Selective/differential media ("infected" milk or yogurt); A1 due
6R	Oct 5	3-12	[Read results]; Milk exercise analysis; A3 due
7T	Oct 10		Practice/Complete Assignments
7R	Oct 12		Practice/Complete Assignments Case Introduction; A4 due
8T	Oct 17		Case discussions/plans Specimen (group) work up
8R	Oct 19		[Read results]; Individual unknown culture work up; [A5]
9T	Oct 24	5-4; 5-2; 5-20	[Read results]; Catalase; Sugar fermentation (mannitol); Antimicrobial susceptibility
9R	Oct 26	5-3; 5-17; 5-10	[Read results]; Voges-Proskauer; PYR; Starch

10T	Oct 31	5-6; 5-8; 5-22	[Read results]; Nitrate; Decarboxylation; CAMP
10R	Nov 2	6-5	Differential WBC
11T	Nov 7	8-4	[Read results]; Latex Agglutination Assays; Repeat or request additional tests
11R	Nov 9		[Read results]; Repeat of request additional tests (final day to request/repeat biochemical tests)
12T	Nov 14	7-2	Kirby-Bauer Antibiotic Susceptibility Assay
12R	Nov 16		Attend Research Forum/Celebration of Scholarship; Last day to submit assignments
13T	Nov 21		[Read results]; ELISA: Outbreak Detection & Analysis
13R	Nov 23		Labs do not meet: Thanksgiving Break
14T	Nov 28		Case Reports Due (beginning of lab); Check out
14R	Nov 30		Labs do not meet: preparation for construction
15T	Dec 5		Labs do not meet: preparation for construction
15R	Dec 7		Labs do not meet: preparation for construction

* Assignment due dates: date at which the first attempt must be made to complete the assignment. You may continue to repeat assignments (A1 – A4) until full credit is earned [last day for assignment assessment is November 16th]

Laboratory Safety

As you will see in one of our exercises, microbes are found everywhere within the environment – in the air, on surfaces, on your body. In the Microbiology 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 in the previous section 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 brief 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 lab coat, 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!
11. SDS (Safety Data Sheets) are in Bolin 223 in the white bookcase

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 squirt bottles of disinfectant (Cidecon) located on the lab benches. You must clean the lab bench before AND after each lab. If you 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.