BIOL 2144: Fundamental Clinical Microbiology Laboratory (Tuesday/Thursday 1:00 – 2:20 pm)

General Information:

Lab Meeting Times; Location: TR (1:00 – 2:20 pm); Bolin Hall 233
Instructor: **James Masuoka, PhD**
E-mail: james.masuoka@msutexas.edu
Office/Tutoring Hours: TR: 9:30 – 11:00 am
WF: 1:00 – 2:00 pm
(Other times by appointment)

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: Essentials by L Norman-McKay, MJ Leboffe & BE Pierce. 2nd Edition. Morton Publishing Company, 2019. ISBN: 978-1-64043-400-4

In addition to the print version, you may access the digital version of the lab manual through Top Hat. The link to Top Hat is in the "Laboratory Resources" folder in the lecture D2L page.

Other Materials (Recommended):

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, also apply to 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. All quizzes, exams, or assignments missed due to unexcused absences will be recorded as zeros.
10. 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.
11. If you have a documented disability that will impact your work in this class, please contact the TA or instructor to discuss your needs.
12. The instructor considers this classroom to be a place where you will be treated with respect as a human being. Students will show respect for each other and the instructor. Failure to do so will result in the disrespectful student being asked to leave the classroom or laboratory. Furthermore, guns or other weapons create a coercive environment that is neither safe nor conducive to learning. Therefore, weapons of any kind will not be allowed in the laboratory. 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.
13. The instructor reserves the right to amend these rules as needed throughout the term.

Attendance Policy:

Students are expected to attend all meetings of lecture and lab. Although in general students are graded on intellectual effort and performance rather than attendance, absences lead to lower overall grades and demonstrate a failure to give priority to your studies. Instructor's records will stand as evidence of absences. A student with excessive absences may be dropped from a course by the instructor. The instructor must give the student a verbal or written warning prior to being dropped from the class (Student Handbook, p. 79).

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

It is the responsibility of the student to obtain notes or other information covered in class during an absence.

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

Grading:

Quizzes:	100 points
Assignments:	60 points
Unknown/Case report:	40 points
Forum Evaluations:	30 points
Participation/safety:	70 points
Total:	300 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 **3 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 should record 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.

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 presented 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 26		**Labs do not meet**
1R	Aug 28		**Labs do not meet**
2T	Sept 2	Introduction	Check-in, Laboratory Safety
2R	Sept 4	9	Ubiquity
3T	Sept 9	3 (A, B, C); 1	[Read results]; Aseptic Technique; Microscope Use
3R	Sept 11	4; 2	[Read results]; Examine Eukaryotes (prepared & wet mount); Streak plate (from Ex 9)
4T	Sept 16	2C	[Read results]; Examine Bacteria (prepared & wet mount) (from streak plate)
4R	Sept 18	11; 13	Making smears; Gram stain (from streak plate)
5T	Sept 23	Handout	Koch's Postulates experiment: milk/yogurt (wet mount, streak plate)
5R	Sept 25	Handout; 4; 13	Inoculate milk (from streak plate) Gram stain (from streak plate)
6T	Sept 30	4; 22; 23; 24; 25	[Read results]; Streak plate ("infected" milk) Selective/differential media ("infected" milk or yogurt)
6R	Oct 2		[Read results]; Milk exercise analysis
7T	Oct 7		Practice/Complete Assignments*
7R	Oct 9		Practice/Complete Assignments* Case Introduction
8T	Oct 14		Case discussions/plans Specimen (group) work up
8R	Oct 16		[Read results]; Individual unknown culture work up
9T	Oct 21	22, 23, 24, 25, 13, 6	[Read results]; Selective/differential media; Gram stain; Colony morphology
9R	Oct 23	29, 36, 28, 32, 18	[Read results]; Catalase; Indole; Methyl Red/Voges-Proskauer; Citrate; Thioglycollate

10T	Oct 28	27, 38, 36, 31, 33	[Read results]; Mannitol fermentation; Lactose fermentation; Coagulase; Motility; Nitrate; Amino Acid Decarboxylation;
10R	Oct 30		**Texas-ASM: TBA**
11T	Nov 4	Handouts	[Read results]; Latex Agglutination Assays; Repeat or request additional tests
11R	Nov 6		[Read results]; Repeat of request additional tests (final day to request/repeat biochemical tests)
12T	Nov 11	43	Kirby-Bauer Antibiotic Susceptibility Assay
12R	Nov 13		[Read results]; **Last day to submit assignments**
13T	Nov 18	See D2L	[Read results]; Differential WBC
13R	Nov 20		**Attend Research Forum/Celebration of Scholarship**
14T	Nov 25	See D2L	ELISA: Outbreak Detection & Analysis
14R	Nov 27		**Labs do not meet: Thanksgiving Break**
15T	Dec 2		**Case Reports Due (beginning of lab)**
15R	Dec 4		**Check out; course evaluations**

*** Assignment due dates: date at which the first attempt must be made to complete the assignment. You may continue to repeat assignments (A1 – A3) until full credit is earned [last day for assignment assessment is November 13th]**

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.