

Microbial Physiology Lab Fall 2021

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LABORATORY: Biology Building, Room B411
LAB TEXT: Lab manual posted on Blackboard

LAB OBJECTIVES

A mixture of bacterial colony morphologies grew on a petri dish. The objective of this lab is for each group of students to utilize microbiological techniques in order to identify selected bacterial colonies. You will use common and advanced lab techniques to examine the physiology of the cell and the many processes the cell uses to produce energy and the metabolites required for survival. You will examine a multitude of techniques, including the principles behind, the uses for, and interpretation of these and other techniques. Understanding this information will be essential to your performance in this class.

LAB GOALS

1. Learn the fundamental concepts in microbial physiology
2. Apply the concepts you've learned
3. Extrapolate information and facts from what you already know
4. Communicate your understanding of microbiology
5. Learn to think critically

LABORATORY EXERCISES

The laboratory exercises are designed to teach fundamental microbiology techniques, including sterile technique, bacterial isolation and culture, macromolecule purification, and identification of microorganisms. The laboratory will meet in Room B412 of the Biology Building, starting the second week of class. The first session will include a REQUIRED laboratory safety lecture. NO ONE will be allowed to work without this training.

LABORATORY POLICIES

SAFETY. Rules exist for your safety, since the organisms we will be using in the lab can make you sick. The first laboratory meeting will include a REQUIRED safety lecture. You will not be permitted to work in the lab until this training has been completed. For Lab PPE, you are **required to provide your own lab coat**, which you must bring to the lab EACH WEEK and **masks**; gloves will be provided. Only closed-toed shoes will be

permitted and long hair must be pulled back OR placed in a hairnet. If you do not meet the dress requirements you will be asked to leave. No food, drink, or application of cosmetics will be permitted in the laboratory.

MISSING LAB. You will only receive credit for a lab report **if you attended the lab itself**. Do NOT miss lab, attendance will be taken each week and a quiz given at the start of class. Do NOT be late for class; excessive tardiness may result in not being given the quiz at the TAs discretion. Since there is only one lab section you cannot make up the lab nor the quiz after it has been given. If you miss a lab for a reasonable excuse, notify your TA. Going to the doctor is NOT a reasonable excuse!! Schedule your appointment for another time. Lab meets at the same time every week and cannot be made-up. **DO NOT** schedule **anything** during this time.

LATE LAB REPORTS. Each lab report is due ONE WEEK after the laboratory exercise has been concluded. It must be turned in to your TA at the beginning of lab. Lab reports will NOT be accepted on any other day. Failure to turn in a lab report will result in an automatic zero.

COURTESY. As a courtesy to your classmates, please give your full attention to all speakers and limit your in-class discussions to topics related to microbiology. Cell-phones and pagers must be turned off during class sessions and labs. The use of laptop or notebook computers or tablets during class and lab sessions is limited to note-taking and coursework only – refrain from browsing the internet or checking your email during class. Please be on time for class – roll may be taken at the start of each class session.

ACADEMIC DISHONESTY. It is the official policy of the University of Texas at El Paso that academic dishonesty is a completely unacceptable mode of conduct and will not be tolerated in any form. Scholastic dishonesty includes, but is not limited to cheating, plagiarism, collusion, the submission for credit of any work or materials that are attributable in whole or in part to another person, taking an examination for another person, any act designed to give unfair advantage to a student or the attempt to commit such acts. All persons involved in academic dishonesty will be disciplined in accordance with University regulations and procedures. Please see <http://www.studentaffairs.utep.edu> for details

DISABILITIES AND MEDICAL CONDITIONS. If you have or suspect you have a disability and need an accommodation, please contact the Center for Accommodations and Support Services (CASS) at 747-5148, at cass@utep.edu or go to Union Building East, Room 106. **If you have medical condition and cannot attend lab in person you need approval from CASS.**

GRADING SYSTEM

Your grade will be based on 7 lab reports, 9 quizzes, midterm and final exams. It is recommended that you maintain a notebook. However, this is for your own study purposes and will not constitute a grade in the class. Lab reports are required and will be due the following week after the lab.

1. QUIZZES. You must read each day's lab manual assignment BEFORE COMING TO LAB. The quizzes are designed to test your knowledge of the material presented in lab and in your reading assignments. Your TA will give the quiz at the beginning of each laboratory period, so please be on time to lab or you will miss that day's quiz. A total of 10 quizzes will be given. One quiz will be dropped and the best 9 counted towards your grade.

2. LAB REPORTS. Lab Reports must be turned in ONE WEEK after each lab session. EACH GROUP will be required to turn in ONE common Lab Report.

3. EXAMS. Two exams will be given during the laboratory time. The exams are worth a total of 100 points. The exam will test your understanding of all of the materials covered in the laboratory and your ability to APPLY the concepts you have learned.

In summary, the grading policy is as follows:

7 lab reports 20 pts each	140 Points
9 quizzes 10 pts each	90 Points
Midterm exam	50 Points
Final	<u>50 Points</u>
	330 Points Total

Materials for this lab:

Lab Coat (Required)

If you do not have a lab coat, you will not be allowed in the laboratory and there are no make-ups for the labs.

Masks

Sharpie (Required)

Notebook (*Not* required but **HIGHLY** recommended)

Lab Manual

The lab manual is available on Blackboard. All protocols will be discussed in the lab prior to the lab in which the protocol is performed. It is recommended that you perform all protocols on paper, prior to lab and before attempting the protocol in lab. This will give you an idea of where and what kinds of problems to expect in lab. This is also a good way to learn the protocols and to understand where the theory of these protocols applies.

LABORATORY SCHEDULE

Lab Report	Series starts week of	TOPICS	Lab report due week of
1	8/29	LAB & BIO SAFETY – REQUIRED Isolation of bacterial colonies	9/17
	9/12	Antibiotic susceptibility testing	
2	9/19	DNA extraction	10/8
	9/26	PCR & Gelectrophoresis	
	10/3	Protein extraction Bradford assay	
3	10/10	Mass spectrometry	10/15
4	10/17	Midterm	10/22
--	10/24	Lipid Extraction & Thin layer chromatography	
5	10/31	Fermentation	11/26
6	11/7	Feedback Inhibition	11/12
7	11/14	Review	12/3
	11/21	Thanksgiving	
--	11/28	Lab final	

BASIC LABORATORY SAFETY

The laboratories planned for this semester are safe. However, we will be working with physical and chemical hazards that require safe handling conditions. Please take a few minutes to review these practices. In the future, as you progress in your scientific training, you will be faced with handling HAZARDOUS chemicals and substances. Therefore, it is essential that you develop good laboratory practices now. In general, if you are not sure of something, ask your instructor! It may prevent a problem.

Carelessness and ignorance are the most common causes of personal injury in the laboratory. It is essential that a student follow the instructions given by instructor.

GOOD LABORATORY PRACTICES RELATED TO YOUR PERSONAL SAFETY:

1. Familiarize yourself with the health and safety hazards of the equipment and chemicals with which you are working. Chemical manufacturers provide a Safety Data Sheet (SDS) for each chemical; these are available for your review. The SDS describes potential hazards associated with working with a substance and gives the emergency response procedures. The SDS should be reviewed prior to the laboratory.
2. Handle hazardous chemicals carefully. Do not move them around the room uncovered. Place them towards the back of the lab bench so there is less chance that they will be knocked over. Never return unused chemicals to the stock bottle. Do not use unlabeled chemicals.
3. Do not taste or inhale any material. Work with chemicals in the fume hood if appropriate (your instructor will note when this is the case).
4. You should wear appropriate clothing to the lab. You MUST wear closed-toed shoes – no sandals, flip-flops, or similar footwear is allowed. If a chemical is spilled or something is dropped on the floor, your foot will be protected. Personal Protective Equipment (PPE) must be worn where appropriate, as indicated by your instructor.
5. If you have long hair, tie it back so that there is no chance it will interfere with your work or get contaminated by a chemical and/or catch on fire.
6. Familiarize yourself with the eyewash station and its use, as well as the locations of fire extinguishers and other emergency equipment and of the exits.
7. Never pipet by mouth!
8. Do not eat or drink in the lab.
9. Remove any PPE before leaving the lab.
10. Wash your hands before leaving the lab. It is very easy to carry small amounts of potentially hazardous chemicals or biological materials on your hands. If you then proceed to eat lunch or dinner without washing your hands, you could ingest these substances unknowingly.
11. Keep work areas clean. Do not pour any chemical down the sink drains. Dispose of chemicals only in labeled containers designated for their disposal.

EMERGENCY RESPONSE PROCEDURES

1. If you are injured, notify the instructor IMMEDIATELY.
2. If a chemical or biological spill occurs, notify the instructor IMMEDIATELY.
3. If the fire alarm is activated, IMMEDIATELY leave the building with your instructor and await further instructions.
4. Emergency contact numbers:

University Police	ext. 5611	3118 Sun Bowl Drive
Main Office, Biological Sciences Department	ext. 5844	Bioscience Research Bldg., Rm. 2.120
Environmental Health and Safety	ext. 7124	Carl Hertzog Building, Rm. 170
Facilities Services Emergency	ext. 7187	3121 Sun Bowl Drive
Life Threatening Situations	911	

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BIOSAFETY PRACTICES

Laboratory Biosafety Levels

The CDC and NIH describe four biosafety levels (BSL) for activities involving infectious agents. The levels are designated in ascending order by degree of protection provided to lab personnel, the environment, and the community. BSL1 is for work with infectious agents that pose minimal or no hazards, while BSL4 is for work with the infectious agents that pose the greatest hazard. Each level recommends facility design, lab practices, and safety equipment appropriate for working with the infectious agent involved. BSL1 through BSL4 are discussed briefly below. A more exhaustive discussion of biosafety level criteria can be found in CDC/NIHs "Biosafety in Microbiological and Biomedical Laboratories." The pathogens studied in the Pathogenic Microbiology (MICR 3144) wet labs are Biosafety Level 1 and Biosafety Level 2 organisms. However, you will use BSL-2 practices and procedures.

Biosafety Level 1 (BSL-1)

BSL-1 practices, safety equipment, and facilities are appropriate for undergraduate teaching laboratories using microorganisms not known to cause disease in healthy adult humans. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended.

Biosafety Level 2 (BSL-2)

BSL-2 practices, safety equipment, and facilities are recommended for clinical, diagnostic, research, or teaching laboratories involving moderate risk agent associated with human disease of varying severity. The primary hazards to lab personnel working with these agents include accidental skin or mucous membrane exposures, or ingestion of infectious materials. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. Primary barriers recommended include biological safety cabinets (BSC) and personal protective equipment (PPE). Secondary barriers recommended include waste decontamination facilities.

Biosafety Level 3 (BSL-3)

BSL-3 practices, safety equipment, and facilities are recommended for clinical, diagnostic, research, or teaching laboratories involving indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Primary hazards to lab personnel working with these agents include autoinoculation, ingestion, and exposure to infectious aerosols. Primary barriers that must be used include BSCs or other enclosed equipment. Secondary barriers for this level include controlled access to the laboratory, a specialized ventilation system, and waste decontamination facilities.

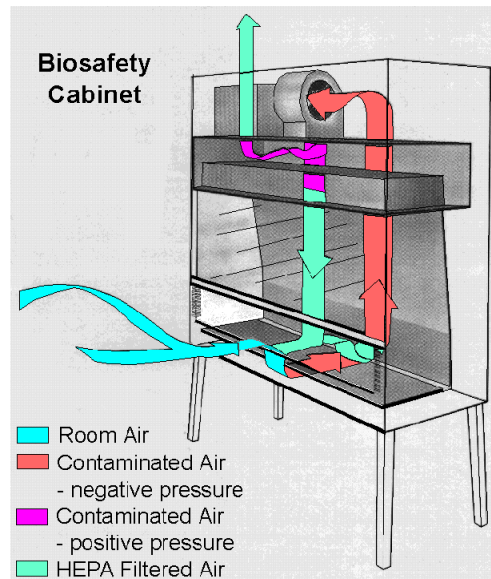
Biosafety Level 4 (BSL-4)

BSL-4 represents maximum containment and is required for dangerous and exotic agents that pose a high risk of life-threatening disease. These are agents that may be transmitted via the aerosol route and for which there is no available vaccine or therapy.

Biological Safety Cabinets (BSC's)

Biological safety cabinets (BSC's) are among the most effective, as well as the most commonly used, primary containment devices in laboratories working with infectious agents. The BSC is designed to capture and contain any infectious particulates or aerosols generated within the BSC's interior and exhaust them through a high-efficiency particulate air (HEPA) filter either into the laboratory, or to the outside.

The three general types of BSC's are available (Class I, II, and III). More detailed information on BSCs can be found in CDC/NIHs "Biosafety in Microbiological and Biomedical Laboratories." The BSC's available to us in Room B412 of the Biology Building are Class II, Type A2. The Class II BSC has an increased face velocity relative to the Class I BSC and the additional advantage of providing protection to the research material by HEPA filtration of the airflow into the cabinet across the work surface. This type of cabinet will protect the user, environment, and the research material and is suitable for work with moderate- to high-risk agents (in the case of our laboratory, Biosafety Level 2). Class II BSCs are classified into two types: A and B. Type A cabinets like ours exhaust the cabinet air into the laboratory. Since the air is re-circulated within the laboratory, volatile or toxic chemicals and radionuclides should not be used inside this type of cabinet. A diagram of airflow circulating in a BSC is pictured below.



Proper use of biological safety cabinets

As with any other piece of laboratory equipment, personnel must be trained in the proper use of the BSC. Of particular note are those activities that may disrupt the inward directional airflow through the work opening of the Class II cabinets. Repeated insertion and withdrawal of the workers' arms into and out of the work chamber, opening and closing doors to the laboratory, improper placement or operation of materials or equipment within the work chamber, or brisk walking past the BSC while it is in use are demonstrated causes of the escape of aerosolized particles from within the cabinet. Strict adherence to recommended practices for the use of the BSC is important in attaining the maximum containment capability of the equipment and maintaining mechanical performance of the equipment itself.

The following steps are essential for proper use of pathogenic organisms in the BSC:

1. Operate the blower in the BSC for five minutes before you begin work in the cabinet to purge any air-borne contaminants.
2. Disinfect the work surface of the BSC before use. A 70% ethanol or a freshly prepared 10% chlorine bleach (sodium hypochlorite) solution is a suitable disinfectant. Keep in mind that the bleach solution is corrosive and can damage the stainless steel work surface. Therefore, it must be followed by a thorough wash with water to prevent corrosion.
3. Set up your work surface. You will need a small biohazard bag for solid waste and a beaker or other vessel (containing your disinfectant) for liquid waste. Keep a squirt bottle of your disinfectant handy in the event that a spill might occur. Do not use a Bunsen burner or other source of flame in the BSC. Instead, you will find a ceramic hot plate in the BSC for use in drying your samples onto the slides prior to Gram staining. Place any slides, tubes, or plates needed for the day's experiments in the BSC prior to beginning work.
4. Be careful not to place any objects on the air intake or exhaust grills, as this will disrupt the airflow. Similarly, always conduct your work at least four inches beyond the opening of the BSC.
5. Always wear a lab coat, eye protection, gloves, and other PPE as indicated by your instructor.
6. In the BSC, work slowly and deliberately. Minimize the movement of your hands into and out of the BSC to avoid disturbing the airflow.
7. Spills happen, but with much less frequency if you are working carefully. If you spill ANYTHING, either inside or outside of the BSC, report it to your instructor IMMEDIATELY. He or she will talk you through the proper cleanup for your spill. You will also be taught some basic cleanup skills during the laboratory safety training, but always enlist the help of your TA. If you're unsure of what to do or have any questions, please call Dr. Spencer at extension 8776 or Environmental Health and Safety (EH&S) at extension 7124.
8. Always properly dispose of tips, tubes, wipers, and any other materials used in the BSC, especially those that have come in contact with biological agents.
9. After completing your work inside the BSC, operate the blower for five minutes before to purge any air-borne contaminants.
10. Remove your PPE and thoroughly wash your hands and arms before leaving the lab.

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I hereby acknowledge receipt of notice of the availability of the Microbial Physiology Laboratory safety manual. I agree to access and read the manual and abide by all policies, safety rules, and procedures defined or referenced in the document.

Name and ID number

Date