

عملي - ميكرو

اللاب الأول

Safety in Microbiology Lab
Microbiology Lab Instrumentations

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	Details
Introduction	<p>The laboratory environment is to be regarded at all times as a restricted area. All lab users, staff, or students are likely to be exposed to many different hazards. These include fire, electrical, chemical, and bio-hazards. Therefore, safety in the laboratory is the primary concern, and it is extremely important that the laboratory rules are followed at all times. A microbiology laboratory is a unique environment that requires special practices and containment facilities.</p> <p>The three main elements of safe containment of microorganisms are:</p> <ol style="list-style-type: none"> 1. Good laboratory practices and techniques 2. Safety equipment 3. Facility design <p>If you are not sure about anything, ALWAYS consult one of the academic staff.</p>
Laboratory Biosafety Levels	<p>According to the Centre for Disease Control and Prevention (CDC), there are 4 different levels of biosafety labs, where each one is designated and equipped for the isolation of specific microorganisms according to their infectious hazards.</p>

Biosafety Levels			
Biological Safety Levels	Description	Examples	CDC Classification
BSL-4	Microbes are dangerous and exotic, posing a high risk of aerosol-transmitted infections, which are frequently fatal without treatment or vaccines. Few labs are at this level.	Ebola and Marburg viruses	
BSL-3	Microbes are indigenous or exotic and cause serious or potentially lethal diseases through respiratory transmission.	<i>Mycobacterium tuberculosis</i>	
BSL-2	Microbes are typically indigenous and are associated with diseases of varying severity. They pose moderate risk to workers and the environment.	<i>Staphylococcus aureus</i>	
BSL-1	Microbes are not known to cause disease in healthy hosts and pose minimal risk to workers and the environment.	Nonpathogenic strains of <i>Escherichia coli</i>	

	Details
Precautions: General Behavior/Safety Measures (Rules)	<p>Upon entering the laboratory, coats, books, and other personal belongings should be placed in specified locations and never on benchtops (except for your lab manual).</p> <p>No running or “horse play.” Report any injuries & accidents immediately. Do not clean them yourself; contact the instructor immediately! In case further treatment is necessary.</p> <p>No food and drink consumption and storage in laboratories, laboratory-like areas, and offices where microorganisms are stored.</p> <p>There will be: - No Smoking - No Eating or Drinking - No pencils, fingers, hair, labels, etc., in the mouth, eye, or nose.</p> <p>At the beginning and the end of each laboratory session, benchtops should be wiped with a disinfectant solution.</p> <p>Observe strict personal hygiene. Wash your hands with antimicrobial soap before leaving the laboratory at the end of the session.</p> <p>Do not wash glassware. All glassware used in the Microbiology Lab must be sterilized before it can be washed.</p> <p>Removal of media, equipment, and cultures from the laboratory is prohibited unless so directed by the instructor.</p> <p>Make sure the gas valve is turned off before you leave the laboratory.</p> <p>You must report to your supervisor (checkout) prior to leaving the laboratory setting.</p>
Why is Lab Safety Important?	<ul style="list-style-type: none"> • Lab safety is a major aspect of every lab-based biomedical class. • Lab safety rules and symbols are needed so that students do not injure themselves or their classmates.
Laboratory Clothing	
Laboratory Gowns or Lab Coats:	<p>Lab coats must be worn at all times in the laboratory.</p> <p>Anyone wearing an inappropriate lab coat will not be allowed to work in the laboratory.</p> <p>Lab coats must be of sufficient length to protect the worker (i.e., down to about the wearer's knees), have long sleeves, and elastic or restricted cuffs.</p>
Footwear:	<p>Closed shoes must be worn at all times (Absolutely no flip-flops allowed) in the laboratory. Anyone wearing inappropriate footwear will not be allowed to work in the laboratory.</p> <p>Students MUST not wear sandals or open-toed or canvas shoes because of the constant danger of cuts and infections from broken glass found on the lab floors and the possibility of chemical spills.</p>

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Eyewear:	Safety glasses must be worn for all activities in the laboratory except when using the microscope or computer or at other times as designated by the Instructor.
Handwear:	Laboratory gloves are NOT to be worn in communal areas. Door handles, telephones, computer keyboards and mice, lift buttons, etc., are not to be touched with gloves. Rubber or disposable gloves should be worn when handling/working with: <ul style="list-style-type: none">• Human blood or other body fluids• Dangerous chemicals• Infectious, or potentially infectious materials.
Lab Safety Equipment:	All students and staff working in the lab must be aware of safety equipment and their locations: Common laboratory equipment: <ol style="list-style-type: none">1. Safety Shower2. Eye Wash3. Safety Goggles4. Fire Extinguisher5. Fire Blanket6. Fume Hood or Safety Cabinet.

Safety shower



Eye Wash



Fire Extinguisher



Safety Goggles



Fire blanket



Fume Hoods / safety cabinet



Lab Safety Symbols

Found in textbook:

- Glassware



- Electrical



- Heat



- Animal



- Eye and Face



- Chemical



- Sharps



- Fire



Chemical Hazard Symbols and Definitions



- **Flammable:** Any substance that will burn if exposed to an open flame.



- **Explosive:** A substance that may explode if exposed to heat or flame.



- **Toxic/Poison:** A substance that can lead to death if inhaled, ingested, or absorbed by the skin.



- **Corrosive:** A substance that can destroy or burn living tissue and can eat away at other materials.



- **Irritant:** A substance that causes inflammation upon contact with skin or mucous membranes.



- **Environmental:** Substances that are harmful to the environment. They must be disposed of properly, not washed down the drain.

Waste disposal:

- ❖ All samples must be considered to be **infectious**.
- ❖ Use of “Universal Precautions” handling
- ❖ Never assume any sample is “*safe*”

- First ask your teacher where/how to dispose of waste.
- Never pour anything down the drain unless you are told to do so.
- A waste basket will be supplied for substance that cannot go down the drain



Where to dispose the waste in lab:

- **Regular waste** in black bag 
- **Infected waste** in yellow bag having biohazard sign. 
- **Sharp waste** in Yellow sharp container. 
- **Pathological waste** in Red bag having biohazard sign. 
- **Cytotoxic & Genotoxic Waste** in Yellow bag. 
- **Chemotherapy sharp** Yellow sharp container 

Reasons for proper waste disposal:

- Hazardous chemicals down the drain can lead to pollution of ground water, lakes, rivers, etc.
- Plants and animals will die if they are exposed to hazardous chemical waste.
- Serious health problems will become present in people if hazardous waste finds its way into drinking water.

Equipment	Details
<p>Microbiological Safety Cabinet or Hoods or Laminar Air Flow Chambers</p>	<p>A microbiological safety cabinet is a piece of laboratory equipment that provides a safe working area for people handling material potentially contaminated with pathogens as well as protecting the sample from any external contamination.</p> <p>Classification of Biosafety Cabinet:</p> <p>Class I Biosafety Cabinet: A ventilated cabinet with an inward airflow and outlet HEPA filters. It was previously referred to as the CDC Hood and served a valuable function in its time by protecting personnel and the environment. Because it offers no product protection, it has been essentially obsolete for the past several decades.</p> <p>Class II (types A and B): Laminar Flow Biological Safety Cabinets that protect personnel, products, and environment. They provide inward airflow to protect personnel, downflow HEPA filtered air to the work area to protect the product, and exhaust HEPA filtered air to protect the environment from particulate and aerosol hazards.</p> <p>Class III Cabinet: Essentially a ventilated glovebox. This is a gas-tight chamber operated through sealed gloves which provide a complete barrier between the worker and hazardous material. The glovebox is maintained under negative pressure with HEPA filtered supply air and double HEPA filtered exhaust air.</p> <p>Laminar air flow is based on the flow of air current to create uniform velocity along parallel lines, which helps in transforming microbial culture in aseptic conditions.</p> <p>Uses: It is used in microbial inoculation and isolation studies. In addition, it is utilized for protection of user, samples, and the environment from hazardous contamination.</p>
<p>Incubators</p>	<p>An apparatus in which environmental conditions, such as temperature and humidity, can be controlled, often used for growing bacterial cultures or providing suitable conditions for a chemical or biological reaction. As for temperature, most commonly used at 37°C. Most bacteria grow well under such conditions.</p> <p>Uses: It is used in cultivating, multiplying, and in the characterization tests of microorganisms. This device provides the heat necessary for the growth of microorganisms.</p>
<p>Hot Air Oven or Lab Oven</p>	<p>Hot air ovens are electrical devices used in sterilization. In sterilizing instruments for various aseptic procedures, especially if that cannot be autoclaved (Dry heat sterilizer) like powders. Generally, they can be operated from 50 to 300 °C. There is a thermostat controlling the temperature.</p> <p>Uses: For sterilization.</p> <p>These are digitally controlled to maintain the temperature. The standard settings for a hot air oven are:</p> <ul style="list-style-type: none"> - 1.5 to 2 hours at 160 °C - 6 to 12 minutes at 190 °C

Equipment	Details
Autoclave	<p>An autoclave (Moist Heat Sterilizer) is a device to sterilize equipment and supplies by subjecting them to high pressure steam ($\approx 15\text{lbs}$) at 121°C or more, typically for 15 to 20 minutes depending on the size of the load and the contents. It was invented by Charles Chamberland in 1879.</p> <p>Uses: The main purpose of this device is to sterilize materials and media under pressure and steam.</p>
Laboratory Refrigerator and Deep Freezer (-86°C)	<p>Refrigerators are used to cool samples or specimens and maintain temperature for preservation.</p> <p>Uses: The device is used for the storage of the stock solutions, chemicals, kits, and nutrient media that should be maintained at certain temperatures.</p> <p>Uses of Deep Freezer: It is used to store stock cultures in microbiology. It is a device used to store materials which should be kept at low temperatures (cells, tissues, enzymes, proteins, etc.).</p>
Bunsen Burner	<p>The Bunsen burner consists of a vertical metal tube connected to a gas source and producing a very hot flame from a mixture of gas and air let in through adjustable holes at the base.</p> <p>The heat generated from the flame helps obtain the required aseptic condition to culture some microorganisms.</p> <p>Named after the German chemist Robert Wilhelm Bunsen, who contributed to its development, the Bunsen burner was already known to Michael Faraday, who may have created the first design in 1855.</p> <p>Uses: Heating, Sterilization & Combustion.</p>
Inoculation Loop/Needle	<p>Its tip is a wire made of platinum or nichrome (Nickel + Chromium).</p> <p>The wire forms a small loop with a diameter of about 5 mm.</p> <p>The inoculation loop is always sterilized in a flame until it becomes red hot before and after each use. After flame sterilization, the loop must be cooled so that the next cells can touch.</p> <p>Needle: Inoculation of agar slopes/dips (Disposable plastic loops and needles).</p> <p>Uses: To inoculate/dip test samples into culture media for bacterial or fungal cultures, antibiograms, etc.</p>
Centrifuge Machine	<p>A laboratory centrifuge is a piece of laboratory equipment, driven by a motor, which spins liquid samples at high speed. There are various types of centrifuges, depending on the size and the sample capacity.</p> <p>Based on automatic rotor recognition, up-to-date centrifuges have a button for automatic conversion of rpm (rotation per minute).</p> <p>Uses: Separation/Isolating suspensions like serum & plasma from blood.</p>
Magnetic Stirrer or Hotplate Stirrer	<p>A magnetic stirrer or magnetic mixer / Hotplate stirrer is a laboratory device that employs a rotating magnetic field to cause a stir bar (also called "flea") immersed in a liquid to spin very quickly, thus stirring it.</p> <p>Magnetic stirrers often include a hot plate or some other means for heating the liquid.</p> <p>Uses: For mixing and keeping the chemical solutions and mixtures at a certain time and temperature by the help of a magnetic bar.</p>

Equipment	Details
Vortex Mixer	<p>Vortex mixers are designed for mixing solutions in test tubes or small flasks with a circular, oscillating movement.</p> <p>Uses: It is used for mixing. Vortex agitates the solutions in the tube, flask, and so on at a certain speed and duration.</p>
Colony Counter	<p>A colony counter is an instrument used to count colonies of bacteria or other microorganisms growing on an agar plate.</p> <p>Method: It uses a pencil-like rod with a metal tip, which is connected by an electrical connection to the gelled growth medium; when touched to a colony on the plate, the completion of the electrical circuit causes an increment of 1 unit on the readout of the colony counter.</p> <p>Uses: For counting bacterial colonies.</p>
Water Bath	<p>A water bath is laboratory equipment made from a container filled with heated water. It is used to incubate samples in water at a constant temperature over a long period of time. All water baths have a digital or an analogue interface to allow users to set a desired temperature.</p> <p>Uses: Warming of reagents, melting of substrates, or incubation of cell cultures. It is also used to enable chemical reactions to occur at high temperatures.</p>
Sensitive Balance	<p>They are used in precise weighing of small amounts (up to milligrams) of samples and chemicals used for preparing media and stock solutions.</p> <p>Uses: For weighing media and chemicals.</p>
Water Distiller	<p>Distillation is a process of separating the component substances from a liquid mixture by selective evaporation and condensation.</p> <p>Uses: For liquid (water) distillation.</p>
Laboratory Thermometer	<p>A laboratory thermometer is a tool used in laboratories to measure temperature with a high level of accuracy.</p> <p>Uses: For measuring temperature.</p>
Anaerobic Jar	<p>It provides anaerobic conditions for the growth of anaerobic bacteria.</p> <p>Uses: For anaerobic cultivation of bacteria.</p>
Microscope	<p>It is an instrument used to see objects that are too small for the naked eye.</p> <p>There are many types of microscopes. The most common microscope is the light or compound microscope.</p> <p>Microscope Magnifying Oil: Oil Immersion: It is a technique used to increase resolving power of a microscope. This is achieved by immersing both the objective lens and the specimen in a transparent oil of high refractive index, thereby increasing the numerical aperture of the objective lens.</p> <p>Cedar Wood Oil or Immersion Oil: Immersion oil contributes to two</p>

Equipment	Details
	<p>characteristics of the image viewed through the microscope: finer resolution and brightness.</p> <p>Applying Immersion Oil: The applicator rods with a taper-point or ball-end make it easy to apply oil to the positioned slide.</p> <p>The magnifications of the image in a compound microscope are as follows: 10x, 10x, 100x. الجدول</p> <p>Uses: For visualizing minute structures including microbes.</p>

General Information on the Use of the Microscope	
1. Handling the Microscope	Take the microscope out of the cabinet and use both hands to lift it to your work area.
2. Note the Objectives	<p>Note the three objectives:</p> <ol style="list-style-type: none"> 1. low power (10X) 2. “high dry” (40X) 3. oil immersion (100X) <p>Adjust the eyepieces in order to use both eyes when viewing specimens.</p>
3. Using Immersion Oil	<p>Place a drop of immersion oil on the subject before examining it with the oil immersion objective (100X).</p> <p>Swing that objective into place and use the fine adjustment knob to bring the subject into focus.</p> <p>Use only the fine adjustment with oil immersion lenses.</p>
4. Light Adjustment	Higher magnification requires more light, so you may need to increase the amount of light.
5. Turning Off the Microscope	Turn off the light source and set the intensity control to zero.
6. Cleaning the Microscope	<p>Always clean the microscope before returning it to its cabinet, especially the lenses and stage.</p> <p>Wipe any dust or oil off the instrument.</p> <p>Use only lens paper and the cleaning fluid to clean the lenses.</p> <p>Rotate the low power objective into place before putting the microscope back into the cabinet.</p>
7. Inspecting the Microscope	Always examine your microscope carefully at the start of each lab period; report any problems with the microscope to the Instructor immediately.
8. Final Check Before Storage	Check your microscope before the end of each lab period (during which you have used the microscope) to ensure that it has been cleaned and prepared for storage properly.