General Microbiology Lab 1 & 2



Laboratory Safety Rules

Use and Care of the Microscope

Objectives

The student should understand the:

1. Definition of aseptic technique

2. Four basic routes of exposure to microorganisms

3. Biosafety Levels

Aseptic techniques

- 1. Is a procedure used by medical staff to prevent the spread of infection
- 2. Is the process by which a sterile (aseptic) materials (typically food, pharmaceutical) is packaged in a sterile container in a way that maintains sterility

The main precautions of aseptic techniques

- 1. Tie back long hair
- 2. Disinfect the tables with antibacterial cleaner.
- 3. Wear gloves and lab coat.
- 4. When labeling Petri plates, always write on the bottom of the plate.
- 5. When using incinerators, give them sufficient time to warm up before sterilizing loops or needles.
- 6. When inoculating cultures, always sterilize your loop or needle before going into a culture and after transferring it. Sterilize the loop even if you are going back into the same culture again.
- 7. Make sure you let your loop cool first to avoid bacterial killing.
- 8. After removing the lid of a test tube, briefly flame the tube before inserting your inoculating loop, and flame again before replacing the cap.
- 9. Don't leave media open to the air bacteria and fungi in the air can cause contamination.
- 10. Don't over-inoculate, simply touch the loop or needle to the bacterial growth and obtain a small amount on the loop.
- 11. When streaking onto an agar plate or slant, make sure the loop doesn't break the surface of the agar. A gentle gliding motion is all that is necessary to distribute the bacteria on the plate

At the end of each lab exercise:

- 1. Remove gloves inside out and place them in the biohazard bag
- 2. Remove lab coats, fold them inside out, and place them in the assigned drawer
- 3. Remove goggles (if used) and clean with alcohol wipes before placing in cabinet for sterilization
- 4. Decontaminate your work bench by applying an antiseptic wash
- 5. Hands washing properly.

The four basic routes of exposure to microorganisms and the specific lab safety guidelines to deal with each of these potential routes of exposure are:

- 1. Contact with skin and mucous membranes: can be minimized by wearing protective equipment such as lab coats, gloves, goggles, respirators, and face shields. Students should be prohibited from inserting contact lenses in the lab.
- **2. Ingestion:** can be minimized by prohibiting eating, drinking, or applying cosmetics in the lab.
- **3.** Inhalation: can be minimized by decrease the likelihood of generating aerosols.
- **4. Inoculation**: can be minimized by following rigid protocols for the use and disposal of sharps (needles, slides, broken glass, etc.)

Microorganisms are divided into 4 Biosafety Levels (BSL):

- 1. **BSL-1**: this level is suitable for work with agents not known to cause disease in healthy adult humans. Precautions in BSL-1 labs include general lab safety rules. Working in BSL1 is generally conducted on open bench tops using standard microbiological practices. Examples include non-pathogenic laboratory strains of *Escherichia coli*.
- 2. BSL-2: this level is suitable for work involving agents of moderate potential hazard to personnel and the environment. Additional precaution in BSL-2 including:

✓ Laboratory personnel should have specific training in handling pathogen

✓ Access to the laboratory is limited when work is being conducted

- ✓ Extreme precautions are taken with contaminated sharp items
- ✓ Biological safety cabinets

Examples include *S. aureus, Chlamydiae*, hepatitis A, B, and C, *influenza* A, *Salmonella*, *Mumps*, and *measles*

1. BSL-3: It includes pathogens cause severe to fatal disease in humans but for which treatments exist. Researchers in BSL-3 labs generally wear double gloves, respirators, goggles and disposable surgical scrubs and gowns, and work in biological safety cabinets-III. Examples such as *Yersinia pestis* (causative agent of plague), *Leishmania donovani*, *M. tuberculosis*, SARS (coronavirus), several species of *Brucella*, and rabies virus

2. BSL-4: defined as easily transmitted, very-high risk microorganisms which cause life-threatening diseases because of <u>no available vaccine</u> <u>or therapy.</u> Workers in BSL-4 labs work in impermeable positive pressure "space suits" with an external oxygen supply, and precautions such as chemical showers must be taken before exiting the lab. Examples include Ebola virus, Marburg virus, and Lassa fever virus.

Lab 2

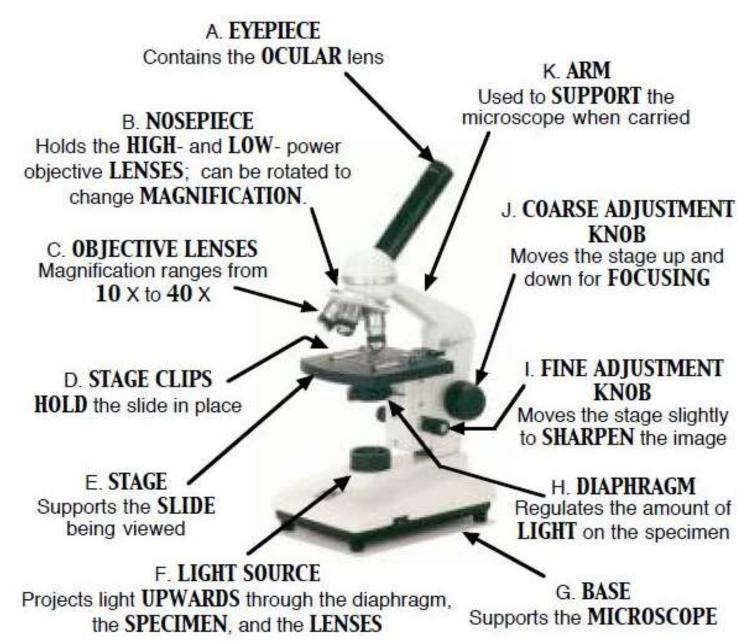
Use and Care of the Microscope

Use and Care of the Microscope

Objectives

- Identify all parts of the light microscope
- Know how to correctly use the microscope especially the oil immersion lens
- Identify the basic morphologies of bacteria by using microscope

Parts of the light microscope



General guidelines for using the microscope:

- Carry the microscope with one hand beneath the base and one hand on the arm.
- Observe the slide with both eyes open, to avoid eye strain.
- Always focus slowly and carefully
- When using the lower-power lens, the iris diaphragm should be barely open so that good contrast is achieved. More light is needed with higher magnification.
- Before using the oil immersion lens, always focus with low power first.
- Keep the stage and lenses clean and free of oil.
- Clean the ocular lenses with lens paper.
- After use:
 - Remove the slid after using the coarse adjustment knob to obtain maximum working distance
 - Wipe oil off from lenses and the stage
 - Put the dust cover on
 - Return the microscope to the designated area