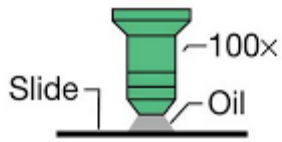
LAB WORK 5.

Subject: Morphology ofthe bacteria.
Session Purpose: Acquaintance with morphology of bacteria and methods of its studying under a microscope fixed stained smear.
Objectives:
1. How to Prepare & Heat Fix a Bacterial Smear for Staining
2. Practice simple staining of bacteria.
3. How to use oil immersion lens.
4. View bacteria under oil immersion.
5. Performexercises 1, 2.

Lab Exercise 1.Stainedfixed smear of rod-shape bacteria.

Methodical instructions: To observe prokaryotic cells, and practice oil immersion techniques, obtain a sample from the source plates or tubes on your lab bench (*Escherichia coli* and *Bacillus subtilis*) according to instructions below. Each lab partner should prepare a slide. Execute aseptic technique, as outlined below, to transfer bacteria to a slide. Observe the cells of rod-shape bacteria (*Escherichia coli* and *Bacillus subtilis*), describe what you see.
Procedure:
1. Use a clean glass slide. Draw two circles with pencil.
2.Put a drop of water in each circle.
3.Inoculate circle of water which each of following: *Escherichia coli*; *Bacillus subtilis.*
4.After heat fixing, stain with Methylene blue and allow to act for 60 sec., rince. **Notice:** No coverslip is required with stained preparations, but take due care when using high magnification that the objective lens does not touch the smear.
5.Then observe under oil immersion lens.
6.Sketch a picture of each type of bacteria under oil immersion
Observing bacteria under oil immersion lens:
1. Make sure that your bacterial smear is clearly in focus at 100 x TM.
2. Put a drop of immersion oil directly on each of the two bacterial smears on your slide, then switch to the oil immersion lens.
3. Only use fine focus adjustment
4. When done, use lens paper to clean up your lens and the stage.



Place drop of oil on slide;
examine with 100× objective

Lab Exercise 2.Stainedfixed smear of sphere-shapebacteria.
Methodical instructions: Prepare a stainedfixed smear of agar bacterial culture.Observe the cells of sphere-shape bacteria (*Staphylococcus aureus* and *Sarcinalutea*), describe what you see.
Procedure:
1. Prepare fixed smears of the two bacterial strains as outlined (*Staphylococcus aureus*; *Sarcinalutea).*

2. Place a slide on the centre supports of the staining rack, and flood the smear with a few drops of the Fuchsine stain, and allow to act for 60 sec. Wash the smear with water (either from a wash bottle or a slow running tap) to remove dye.
3. Dry the slide using absorbent paper pressed lightly over the surface.
4. Examine the stained preparation under oil-immersion lens.
Notice: Remember to wipe the oil-immersion lens with tissue whenever it is removed from the immersion oil.

Proper Storage of Your Microscope

To practice proper care of your microscope, make certain to clean it and put it away properly at the end of this exercise.
1. Make certain the slide is removed from the stage.
2. Clean all lens with Lens Paperif there is excess oil.
a. Obtain a clean sheet of lens paper.
b. Rub oculars to clean as demonstrated.
c. After use, carefully clean all lens surfaces with lens tissue. If by chance oil gets onto the x10 or x40 objectives wipe off immediately. Do not leave immersion oil on the immersion objective when you put the microscope away.
3. Put the scanning power objective in place.
4. Pull the body tube away from the stage (i.e. lower the stage as far as possible)
5. Wrap the cord.
6. Return the proper storage location in the cabinet.

Equipment:

- Microscope
- Slide
- Dropper bottle of water
- Disinfectant tray
- Culture of Bacteria in slant tubes
- Inoculation loop
- Burnerflame
- Staining material - Methylene blue and Fuchsine stains
- Immersion oil