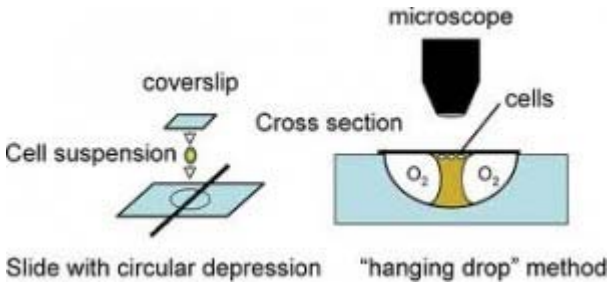
LAB WORK 10.

Subject: Investigating bacterial motility by flagella.
Session Purpose: Motility procedures & testing**.**
Objectives:

1. To observe bacteria in a «hanging drop», study their morphology, and determine their motility.
2. To determine bacterial cultures grown in motility test medium.
3. Performexercises 1, 2.

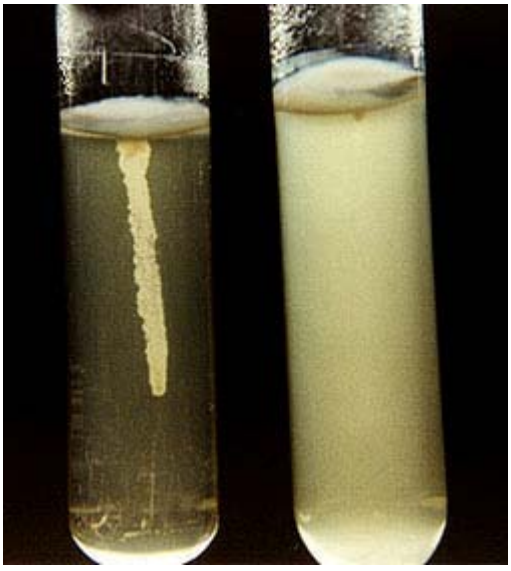
Methodical instructions: The motility of bacteria can be determined by several methods:

1) It can be determined microscopically by observing cells in a **wet mount**. This method is called the **hanging drop technique**. In this procedure a drop of cells is placed on a cover slip which is then placed on a special slide with a concave depression in its center. The coverslip is held in place with petroleum jelly. This creates an enclosed glass chamber that prevents drying. It is important to distinguish between cells that are moving due to the vibrations of the table and microscope and cells that are actually motile.



Hanging drop technique

2) An alternative method, one that is safer when working with potential pathogens, is the **motility stab.** In order to determine if an organism is motile in **semi-soft medium (**SSM), examine the stab line where the tube was inoculated. If an organism is non-motile, it will be concentrated along the stab line. If the organism is motile, uniform turbidity is observed throughout the media. This distribution of cells within the agar is an indication the organism possessed flagella which allowed movement into the media.



Reaction of motile and non-motile microbes in SSM

Lab Exercise 1.«Hanging drop» or a simple «wet mount».

Procedure:

1.Using a toothpick applying a circular "ring" of Vaseline around the edge of depression on the slide.

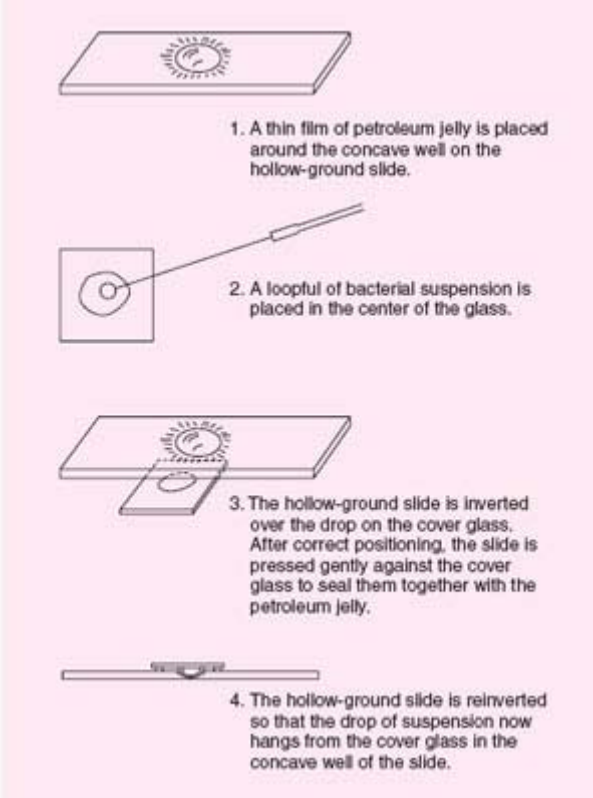
2. Take a cover glass and clean it thoroughly. It may be dipped in alcohol and polished dry with tissue.

3. Using good aseptic technique, sterilize the wire loop, remove the cap of the tube, and take up a loopful of culture. Be certain the loop has cooled to room temperature. Close and return the tube to the rack.

4. Place the loopful of agar culture in drop of water in the cover glass as in figure 3, step 2 (do not spread it around). (Place 5-10 loopfuls of the mixed overnight incubated broth onto a coverslip. Sterilize the loop and put it down.

5. Hold the hollow-ground slide inverted with the well down over the cover glass, then press it down gently so that the petroleum jelly adheres to the cover glass. Now turn the slide over. You should have a sealed wet mount, with the drop of culture hanging in the well.

6. Place the slide on the microscope stage, cover glass up. Make your examination with the high-dry and oil-immersion objectives (be very careful not to break the cover glass with the latter). Reduce your Iris to very LOW LIGHT!Focus under oil immersion and look for "tumbling" behavior.



Hanging drop setup for observing motility under the microscope

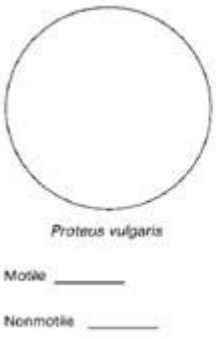
Note:

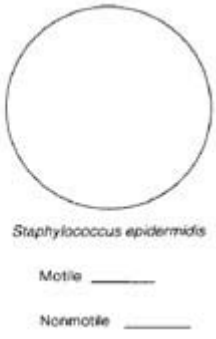
1) To make visualization easier you can be added tothe bacterial suspensiondropof a weak solutionof methylene blue.

2) Vibrating Microbes are NOT motile. You MUST focus within 3 minutes of preparation of the slide as by 5 minutes all the microbes are NON-MOTILE as they are dead - killed from the heat produced by the light source.

Result:

Make drawings in the following circles to show the shape and grouping of each organism. Indicate below the circle whether it is motile or nonmotile.





Lab Exercise 2.The motility gel deep/STAB test.

Procedure:

Period 1.

1. Inoculate each bacterial culture into a separate tube of the semisolid Motility Medium.
2. Use the needle and carefully stab-inoculate the medium about half-way down through the center.
3. The wire is then pulled out of the media as close as possible to the location where it entered.
4. The tube is incubated for approximately 1-2 days at 30 °C and observed for evidence of motility.

Period 2.

1. Observe the tubes of Motility Medium for growth away from the line of inoculation and the subsequent cloudiness throughout the medium as discussed in the introduction. In a well-lit room, hold all of the tubes together against a darker part of the ceiling (such as the space between the fluorescent light units) so that degrees of growth can be discerned easily. Ignore all surface growth and any growth that might be creeping down from the surface along the inner wall of the tube.
2. Tabulate your results. Complete the table. Confirm your Positive Hanging-drop Motility with a Stab Motility Test.

Bacterial cultures grown in motility test medium

Bacterial culture	Motility in Hanging-drop	Motility in Stab Test

Equipment:

- Microscope
- Slide
- Several cover glasses
- Several hollow-ground slides

    - Dropper bottle of water
    - Disinfectant tray
    - Inoculation loop
    - Burnerflame
    - Immersion oil
- Young broth cultures (12-15 hours) of *Enterobacteraerogenes* (or other motile organism) and *Staphylococcus epidermidis*. These will serve as positive and negative controls (respectively) for the unknown.
- Young broth culture of an unknown organism
- Several tubes of semisolid medium such as 0.75% agar