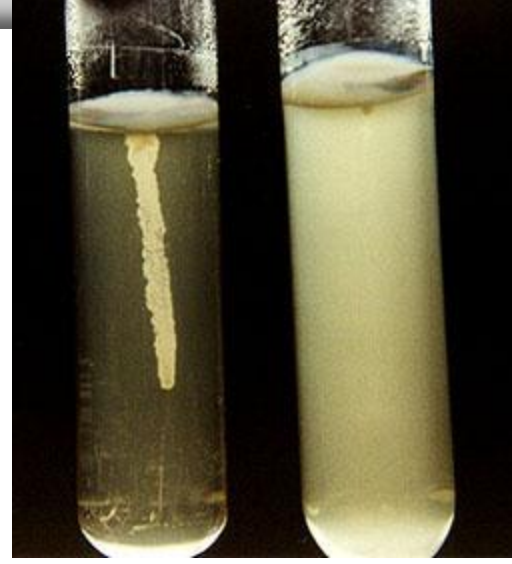




General Microbiology Laboratory



Bacterial motility



Introduction to bacterial motility

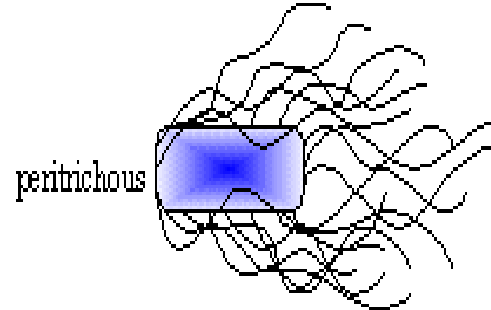
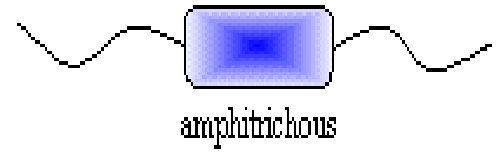
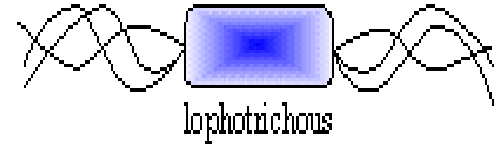
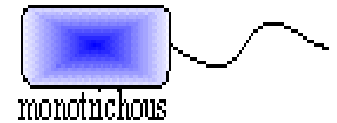


- ❖ A large number of bacteria are motile.
- ❖ Most possess one or more flagella on their surface that allow them to swim.
- ❖ Bacterial flagella are tiny hair like organelles of locomotion. Originating in the cytoplasm beneath the cell wall, they extend beyond the cell, usually equaling or exceeding it in length.
- ❖ The straight line movement is called a run and the turn is called a tumble.
- ❖ Runs and tumbles are controlled by the clockwise or counterclockwise rotation of the basal body of the flagellum, the motor that is anchored in the cell membrane.
- ❖ Their fine protein structure requires special staining techniques for demonstrating them with the light microscope.



❖ The pattern of flagellation is an important feature in identification of motile bacteria.

- Polar flagella occur at one or both ends of the bacterium (*Vibrio cholerae* and some species of *Pseudomonas*). They may be single or in tufts.
- Lophotrichous (spirillae). Peritrichous flagella are distributed around the surface of the organism (many *Proteus* species).
- Most motile bacteria move in a straight line for a brief time, then turn and randomly change directions before swimming again



Motility testing



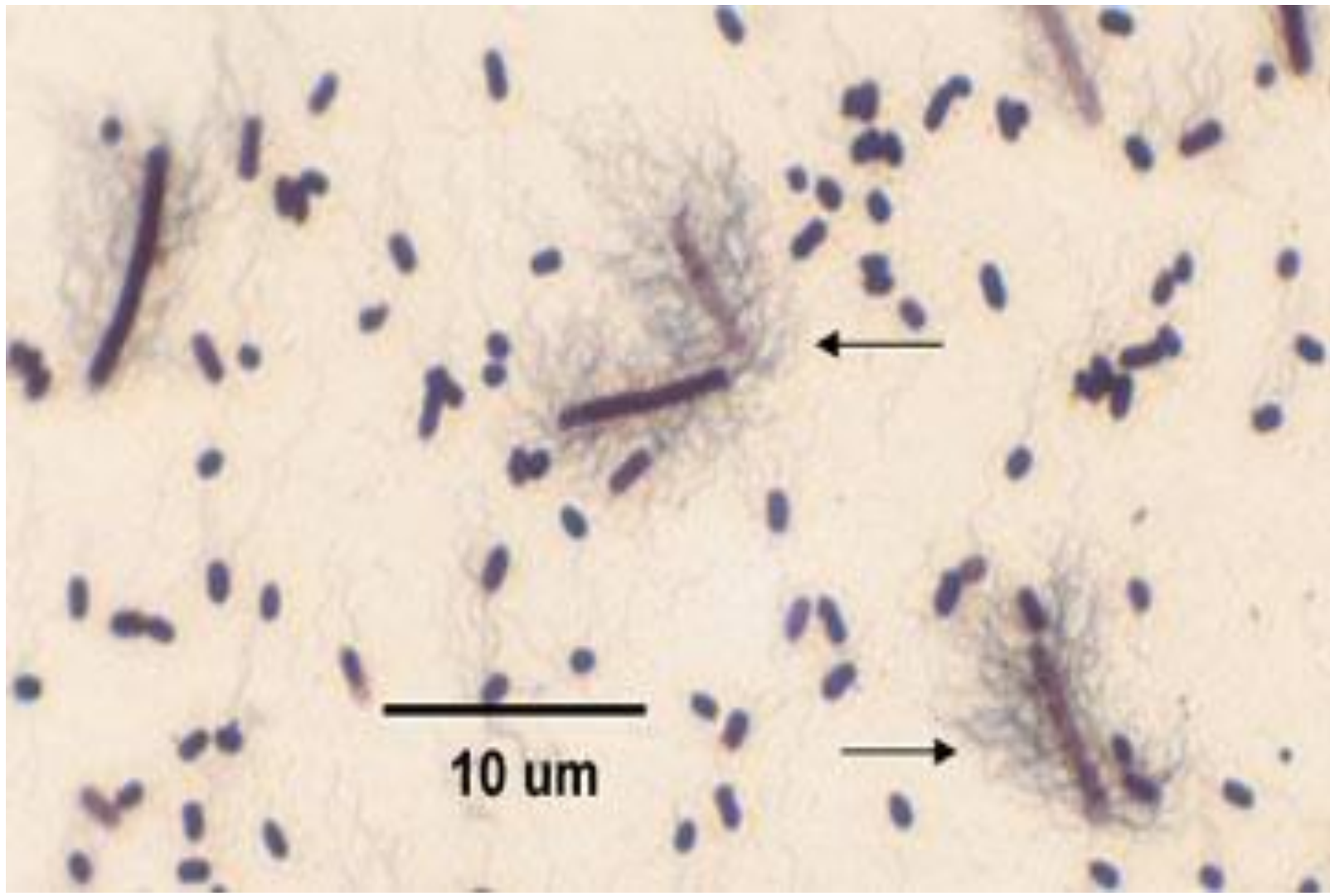
❖ Motility could be detected by:

1. Flagellar stain.
2. Hanging Drop technique.
3. Semi-Solid media Inoculation.

Flagellar stain



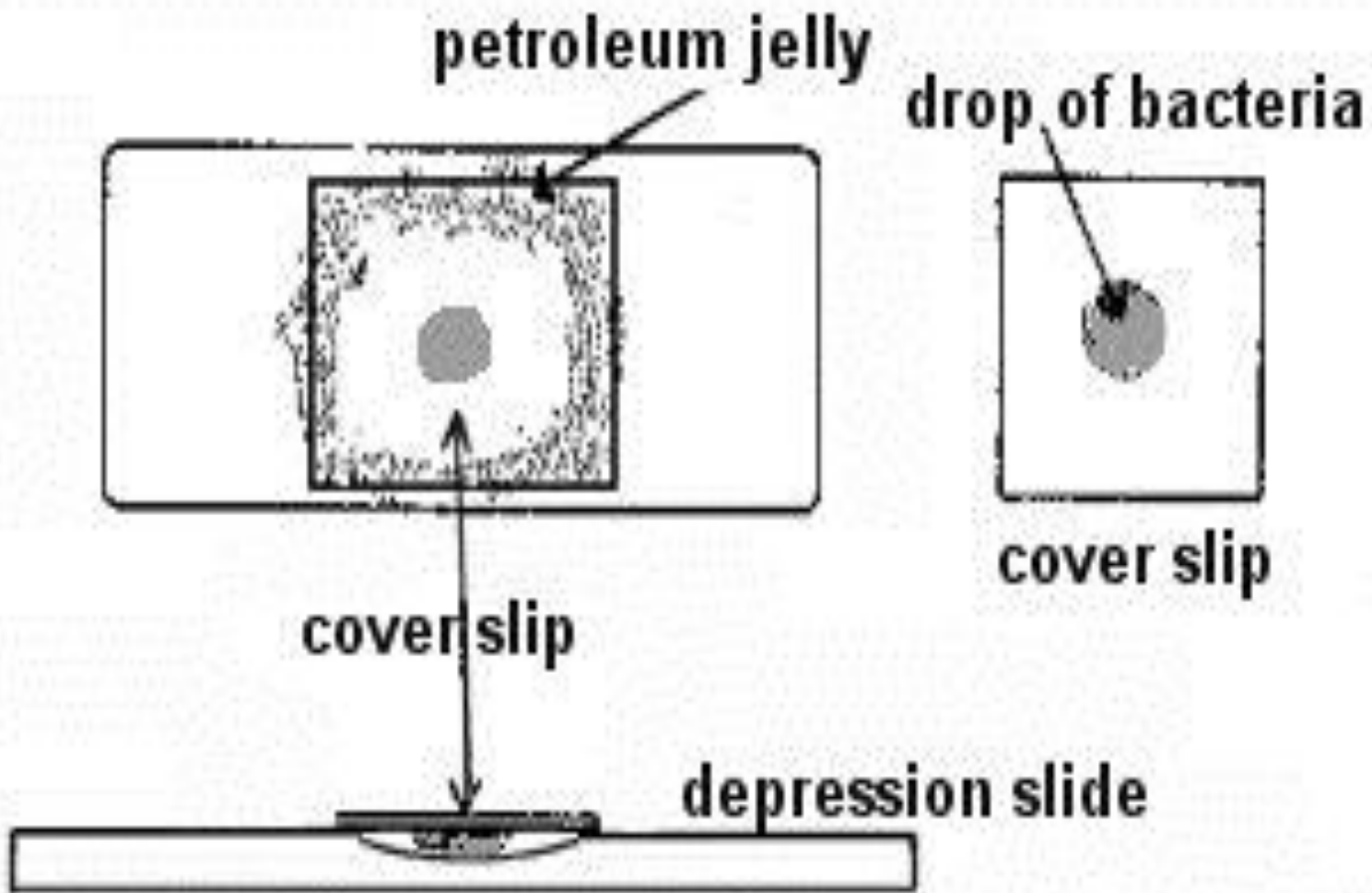
- ❖ Flagella are too thin to be seen by the ordinary light microscope.
- ❖ Flagella should be amplified (enlarged). Use a stain that is specifically deposited on Flagella thus increasing diameter.
- ❖ Some flagellar stains employ **rosaniline dyes** and a **mordant**, applied to a bacterial suspension fixed in **formalin** and spread across a glass slide. The formalin links to, or “fixes,” the flagellar and other surface protein of the cells. The dye and mordant then precipitate around these “fixed” surfaces, enlarging their diameters, and making flagella visible when viewed under the microscope.
- ❖ Another method, a **ferric-tannate mordant** and a **silver nitrate** solution are applied to a bacterial suspension. The resulting dark precipitate that forms on the bacteria and their flagella allows them to be easily visualized under the microscope. This silver-plating technique is also used to stain the very slender spirochetes.
- ❖ The techniques are somewhat sensitive.



Hanging Drop technique



1. Place a drop of the bacterial culture (optimally from a young broth culture) in the middle of a cover slip.
2. Place a thin line of petroleum jelly around the edge of the cover slide.
3. Turn the depression slide upside-down (depressed area facing down) and gently touch the cover slide. The jelly holds the cover slip to the slide and also keeps the suspension from drying out.
4. Now flip the entire microscope slide/cover slip combination over. It should look like the diagram below



Important !!!!!



- ❖ You should be able to differentiate true motility from Brownian motility
- ❖ Brownian movement is usually caused by the activity of water molecules. (characterized by back and forth movement)
- ❖ True motility (the bacterial cells runs and tumble.)

Semi-Solid media Inoculation



- ❖ The most commonly used test for motility in microbiology lab.
- ❖ It depends on the ability of motile bacteria to move through semi-solid media.
- ❖ Ordinary solid media contain 1.5-2.0% Agar
- ❖ Semi solid media contain about 0.4% Agar

Procedure of Motility Test



❖ **How to Perform Test:**

- Using a sterile bacteriological needle, pick a colony of the test organism
- Stab quickly a tube of semi solid media. (avoid using bent needles).
- Incubate the semi solid media for 24 hours.

❖ **Property it tests for:** This test is done to help differentiate species of bacteria that are motile.

❖ **Media and Reagents Used:**

Motility media contains tryptose, sodium chloride, agar, and a color indicator.

❖ **Reading Results:**

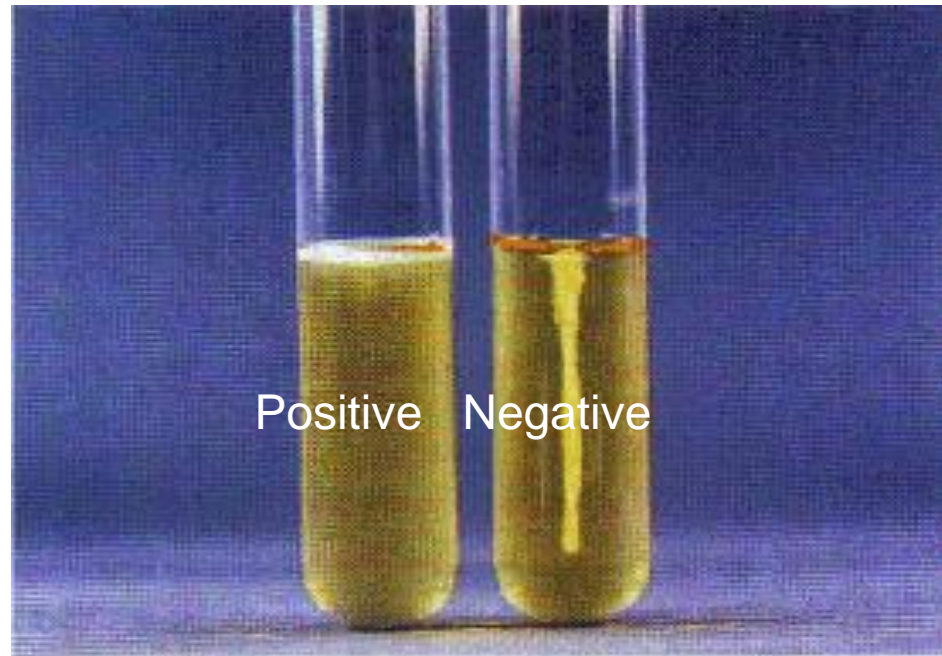
- If bacteria is motile, there will be growth going out away from the stab line, and test is positive.
- If bacteria is not motile, there will only be growth along the stab line.
- A colored indicator can be used to make the results easier to see.

Semi-Solid media results

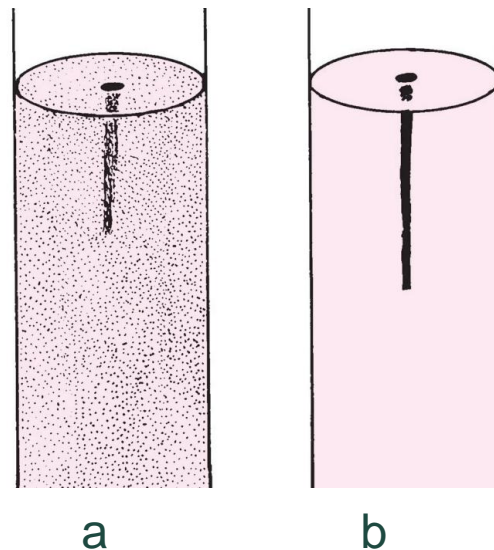


Sketch and photograph of semisolid agar tubes stabbed for motility test.

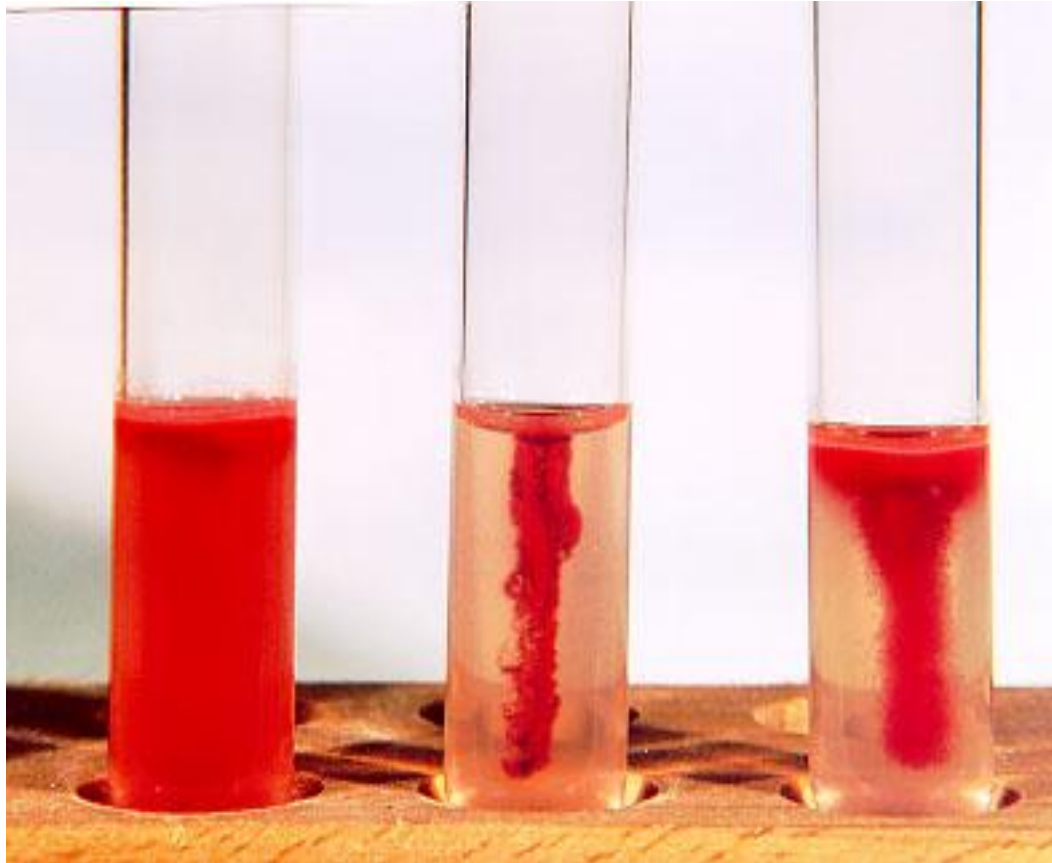
(a) Pattern of growth of a motile organism. The entire medium is turbid with the growth of the organism, which has moved away from the stab line.



(b) Pattern of growth of a nonmotile organism. Only the stab line is turbid with growth.



Semi solid media with tetrazolium chloride (color indicator)



From left to right:

+

-

+



End of lecture