



Determination of moving bacteria and Acid-fast staining procedure

Motility determination:

The ability of an organism to move by itself is called motility. Motility is closely linked with *chemotaxis*, the ability to orientate along certain chemical gradients. Eucaryotic cells can move by means of different locomotor organelles such as cilia, flagella, or pseudopods. Procaryotes move by means of propeller-like flagella unique to bacteria or by special fibrils that produce a gliding form of motility.

Three methods are employed for motility determination depending on the pathogenic capability of the organisms. For nonpathogens, there are two slide techniques that one might use. For pathogens, tube method can be used.

I) Slide methods for non-pathogens include

1. Wet Mount slide
2. Hanging Drop slide

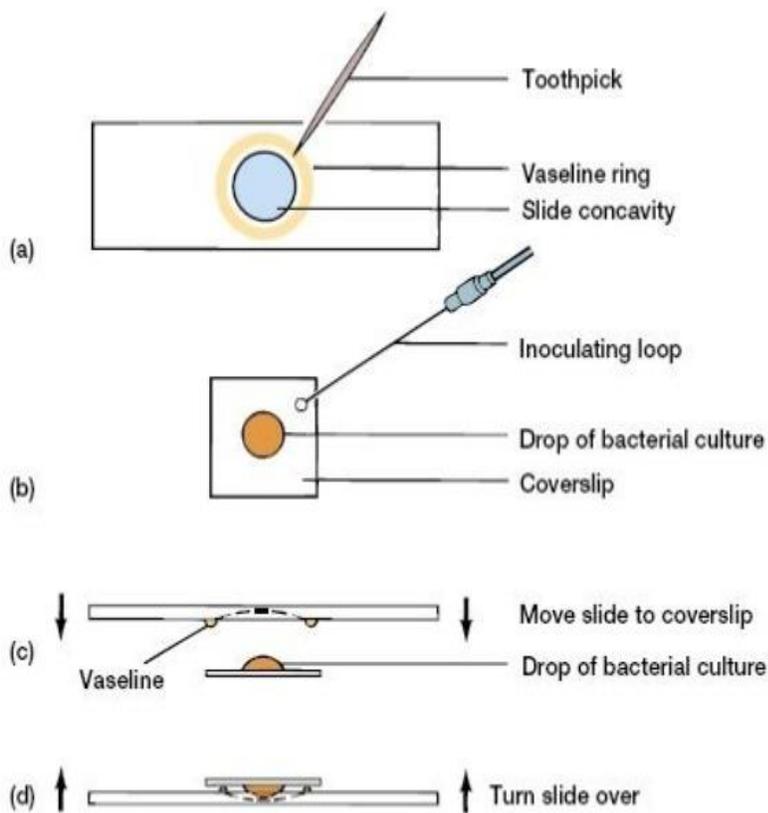
1. Wet Mount slide

When working with nonpathogens, the simplest way to determine motility is to place a few loopfuls of the organism on a clean slide and cover it with a cover glass. In addition to being able to determine the presence or absence of motility, this method is useful in determining cellular shape (rod, coccus, or spiral) and arrangement (irregular clusters, packets, pairs, or long chains).

2. Hanging Drop slide

Hanging drop slides are useful in observing the general shape of living bacteria and the arrangement of bacterial cells when they associate together. Organisms are observed in a drop that is suspended under a cover glass in a concave depression slide. The slide for a hanging drop is ground with a concave well in the center; the cover glass holds a drop of the suspension. When the cover glass is inverted over the well of the slide, the drop hangs from the glass in the hollow concavity of the slide. Since the drop lies within an enclosed glass chamber, drying out occurs very slowly. A ring of Vaseline around the edge of the cover slip keeps the slide from drying out.

Preparation of a Hanging Drop Slide.



II) Method for pathogenic microorganisms includes

Soft Agar Stabbing (Tube Method):

Used to determine the motility of pathogenic microorganisms (such as the typhoid bacillus) by slide techniques. In semi-solid agar media, motile bacteria 'swarm' and give a diffuse spreading growth that is easily recognized by the naked eye.

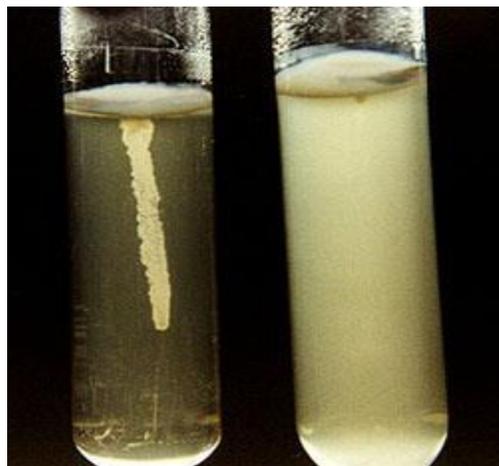
Procedure:

- 1-Prepare a semisolid agar medium in a test tube.
- 2-Inoculate with a straight wire, making a single stab down the center of the tube to about half the depth of the medium.
- 3-Incubate under the conditions favoring motility. Incubate at 37°C
- 4-Examine at intervals, e.g. after 6 h, and 1 and 2 days (depends on generation time of bacteria) .

Results: Hold the tube up to the light and look at the stab line to determine motility.

Non-motile bacteria generally give growths that are confined to the stab-line, have sharply defined margins and leave the surrounding medium clearly transparent.

Motile Bacteria typically give diffuse, hazy growths that spread throughout the medium rendering it slightly opaque.



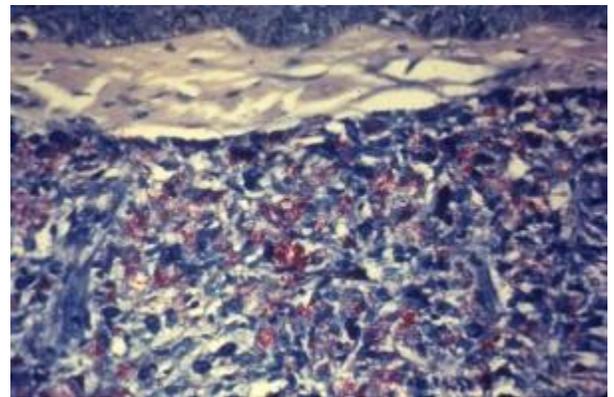
Ziehl Neelsen (Acid Fast) staining

Principle

This procedure is used to stain *Mycobacterium tuberculosis* and *Mycobacterium leprae*. These bacteria are also called acid fast bacilli. They stain with carbol fuchsin, which is a red dye. They retain the dye when treated with acid, which is because of the presence of mycolic acid in their cell wall.

Reagents

1. Carbol fuchsin (basic dye)
2. Mordant (heat)
3. 20% sulphuric acid (decolorizer)
4. Methylene blue (counter stain) or Malachite green



Procedure

1. Fix the smear of the specimen over the glass slide, either by heating .
2. Pour carbol fuchsin over smear and heat gently until fumes appear. Do not overheat and allow it to stand for 5 minutes, then wash it off with water.
3. Pour 20% sulphuric acid, wait for one minute and keep on repeating this step until the slide appears light pink in color. Wash off with water.
4. Pour methylene blue, wait for two minutes, again wash with water
5. Allow it to air dry and examine under oil immersion lens.

Result

-Acid fast bacilli stain pink, straight or slightly curved rods, at times having beaded appearance

-The background appears blue due to methylene blue.

