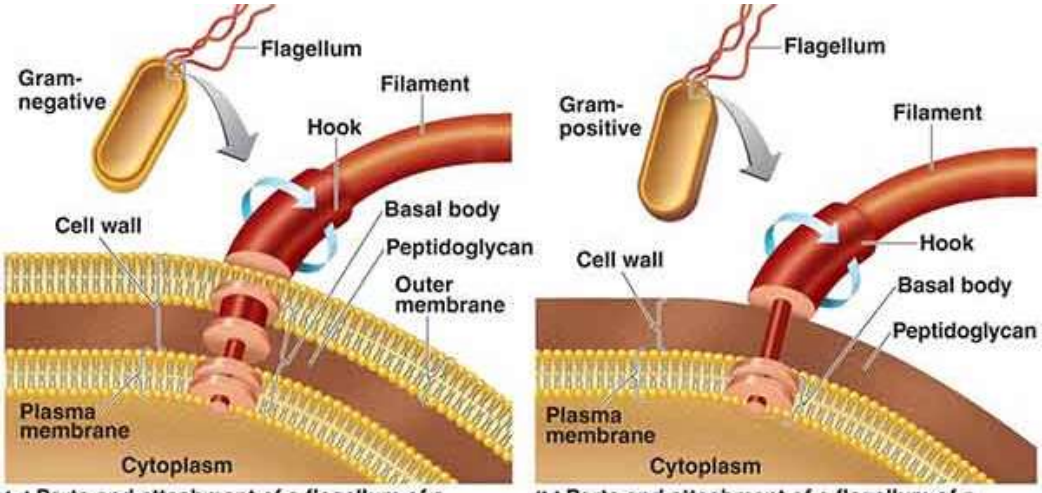
Structural Staining Method
(Flagella staining)

Flagella are filamentous protein structures attached to the cell surface that provide the swimming movement for most motile procaryotes towards nutrients and other attractants. The diameter of a procaryotic flagellum is about 20 nanometers and its length about 15-20 µm. Flagellum can never be seen directly with the light microscope but only after staining with special flagella stains.

Structure of Flagella:

Flagellum consists of a Basal body, Hook and a Main Filament.

- **Basal Body:**
  In gram -ve bacteria, basal ring has 2 sets of rings, Proximal set and Distal set. Each set has 2 rings, and the four rings are M-ring, S ring, P ring, L ring. The arrangement of these rings are from inside to outside.
  M-ring and S ring are anchored to Plasma membrane, P-ring to the Peptidoglycan layer of the cell-wall and the L-ring attached to the Lipopolysaccharide layer.
  M-ring provides the "movement" to the flagella. The S-ring is the Static-ring.
  While in Gram +ve, there are only single set of rings, no attachment to cell wall is seen. The inner rings are attached to the plasma membrane.
- **Hook:** It penetrates the cell-wall, and connects main Filament and Basal body.
- **Filament:** It consists entirely of Protein, called "Flagellin".



(a) Parts and attachment of a flagellum of a gram-negative bacterium

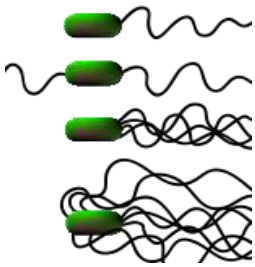
(b) Parts and attachment of a flagellum of a gram-positive bacterium

This motility of bacteria occurs in 2 different phenomena:

1. Phototaxis: movement of bacteria in response to Light. This phototaxis is of two types
   a. positive phototaxis : towards light and
   b. negative phototaxis : away from light
2. Chemotaxis: movement of bacteria in response to chemicals. This Chemotaxis is in two types:
   a. positive chemotaxis: towards chemical and
   b. negative chemotaxis: away from chemicals.

Arrangement and Types of Flagella

The number and location of flagella are distinctive for each genus. There are four types of flagella arrangement.



1. Monotrichous: Single polar flagellum, e.g. *Vibrio cholera*
2. Amphitrichous: Single flagellum at both ends, e.g. *Alcaligenes faecalis*
3. Lophotrichous: Tuft of flagella at one or both ends, e.g. *Thiospirillum jenense*
4. Peritrichous: Flagella surrounding the cell, e.g. *E. coli, Proteus* spp.

Functions of Bacterial Flagella

Many prokaryotes are motile, and the majority of motile prokaryotes move by means of flagella.

Importance of Flagella

1. **Role in Pathogenesis:**
   Escherichia coli and *Proteus* spp are common cause of Urinary tract infections. The flagella of these bacteria help the bacteria by propelling up the urethra into the bladder. It is called **H** antigen.
2. **Roles in Organism identification**
   a. Some species of bacteria, e.g. *Salmonella* species are identified in the clinical laboratory by the use of Specific antibodies against flagella proteins.
   b. Organisms such as *Vibrio cholerae* (darting motility) and *Proteus* species (swarming growth in common culture media) are easily identified by their characteristics motility pattern.

Procedure

A. (Hanging drop preparation method)

Bacterial Suspension (From an agar slant culture):

a. Suspend a loopful of bacterial colonies in 2 ml distilled water to obtain suspension.
b. Allow the suspension to stand undisturbed for 15 to 20 minutes while flagella are regenerated and extended.
1. Hold a clean coverslip by its edges and carefully dab distilled water on its corners using a toothpick.
2. Place a loop full of the bacterial suspension to be tested in the center of the prepared coverslip.
3. Turn the clean concavity slide upside down over the drop on the coverslip.
4. Turn the slide over so the coverslip is on top and the drop can be observed banging from the coverslip over the concavity.
5. Place the preparation in the microscope.
6. Observe the cells noting their morphology and grouping and determine whether true motility can be observed.
7. Brownian movement should be visible on slides of all the organisms, but two should also show true motility.

B. (Semi – Solid media method).

1. Prepare the semi-solid medium by adding 0.3 – 0.5% of the weight of agar which present in the agar medium to broth medium (like nutrient broth).
2. Sterilize by putting into the autoclave at 121 °C for 15 minutes.
3. Take tube 10 ml and add 5 – 7 ml of prepared semi-solid medium.
4. Inoculate the medium with bacterial suspension as in first method.
5. Incubate for 24hrs at 37 °C.