Flagella staining

-Ms. Sanchari Sarkar
Department of Microbiology
Shivaji Science College, Nagpur

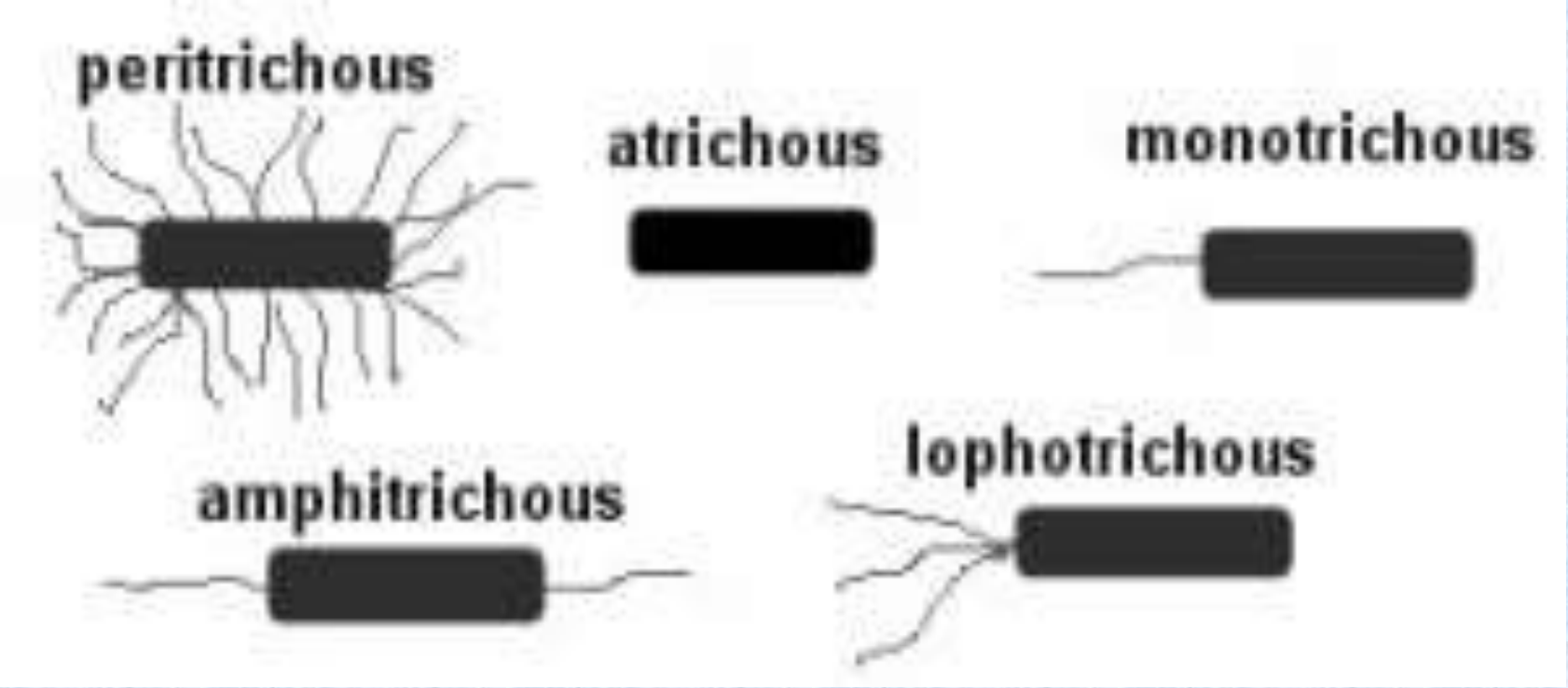
• Flagella are the complex filamentous cytoplasmic structure protruding through cell wall.

• These are unbranched, long, thread like structures, mostly composed of the protein flagellin, intricately embedded in the cell envelope.

• They are about 12-30 nm in diameter and 5-16 µm in length. They are responsible for the bacterial motility.

• Motility plays an important role in survival and the ability of certain bacteria to cause disease.

- Types of flagella:
- Monotrichous
- Lophotrichous
- Amphitrichous
- Peritrichous



- Functions of Flagella :

• Movements
• Sensation
• Signal transduction
• Adhesion
• For cells anchored in a tissue, like the epithelial cells lining our air passages, this moves liquid over the surface of the cell (e.g., driving particle-laden mucus toward the throat).
• Flagella are generally accepted as being important virulence factors

- Flagella is one of the most important locomotory organ .It is mainly made up of three parts- 1) Basal body 2) Filament 3) Hook.
- Flagella is generally present in rod shape bacteria and very few cocci shape bacteria posses flagella.
- As flagella are very thin and hair like they cannot be easily observed under microscope.
- So a special technique is design to increase thickness of flagella as well as stain it.
- Due to these technique we can observe structure of flagella easily under microscope.

- Requirements:

- Flagellated cell culture slant.
- Leifson’s stain.
- 1 % Methylene blue.
- Distilled water.

- Procedure :

- First of all take two hours old flagellated cell culture slant and add two to three drops of sterile distill water in the slant with the help of sterile pipette.

• Note that the distilled water is added slowly without disturbing the growth of cells.
• After addition of distilled water incubated the slant for 20 minutes.
• Then take a drop of suspension from the slant and place the drop on a clean slide which is kept in slanting position.
• The drop should flow slowly from one end of slide to other end to avoid folding of flagella on cell.
• Allow smear to air dry here we don’t use heat fixation treatment .

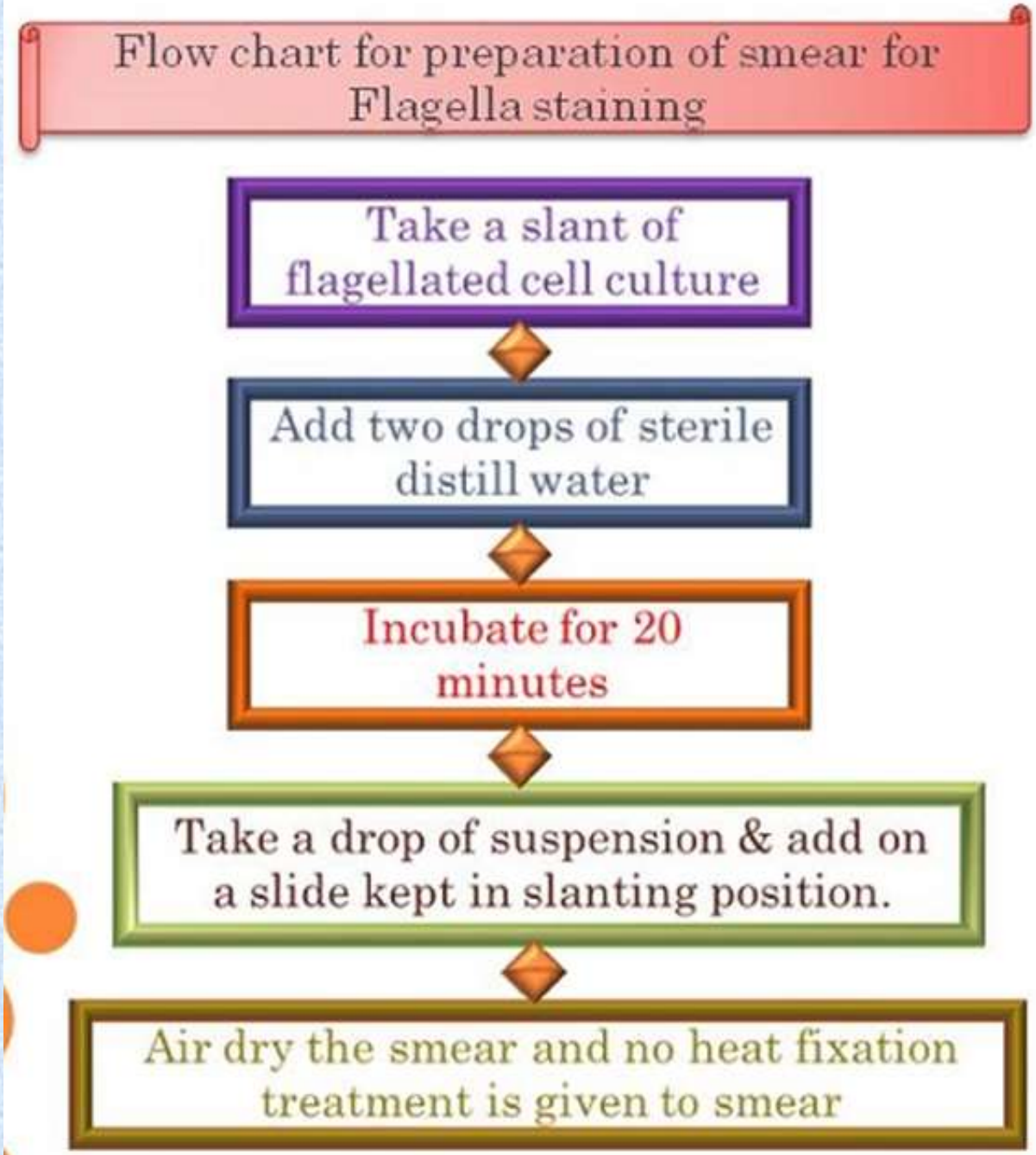
• After air drying the slide is flooded with Leifson’s stain till a thin film of shinny surface appear.

• After this give a gentle stream of water wash treatment to a slide.

• Now treat the slide with 1 % methylene blue treatment for 1 minute.

• Give the slide water wash treatment ,air dry and observe under oil immersion lens.

Flow chart for preparation of smear for Flagella staining




Flow chart of Flagella staining procedure
Prepare a smear on a slide
Flood the slide with Leifson's stain
After appearance of shinny film give a stream of water wash
Treat the slide with 1 % Methylene blue for 1 minute
Wash the slide with water ,air dry and observe under oil immersion

- Mechanism:

• First of all in this procedure thickness of flagella is increase so it can be visible.

• The Leifson’s stain is made up of tannic acid, basic fuschin stain prepared in alcohol base.

• When we treat Leifson’s stain with cell the tannic acid get attach to the flagella and alcohol get evaporated.

• After evaporation of alcohol the thickness of flagella is increased due to deposition of tannic acid. where as Basic fuschin stain the Flagella.
• After Leifson's stain treatment cells are treated with Methylene blue stain. This Methylene blue stains the cell.

- Result:

After observation under microscope we can observe that flagella appear red in colour and bacterial cell appear blue in colour.