DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY

HANGING DROP PREPARATION

Introduction

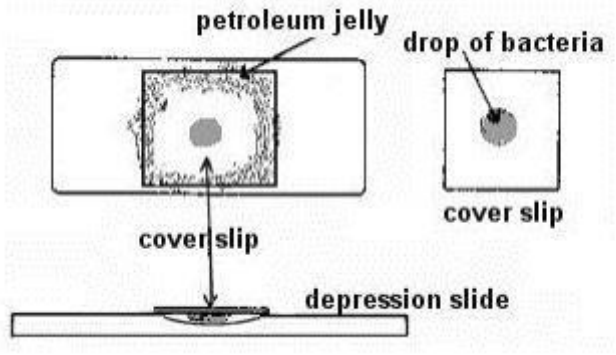
Some bacteria are motile and some other are non-motile. Motile bacteria usually use flagella as their locomotory organ. Bacteria tend to move towards or away from various chemotactic, phototactic, aerotactic or magnetotactic stimuli.. There are various ways to demonstrate bacterial motility. These include, a simple wet mount, hanging drop preparation, or employment of soft gels (semi-solid agar).

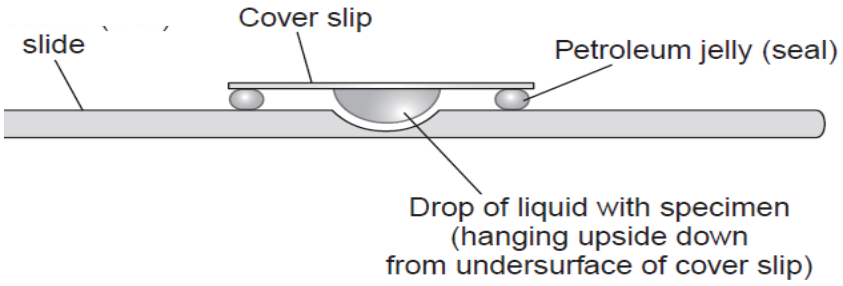
Materials required:

1. Cavity slides (glass slide with depression)
2. Paraffin wax
3. Innoculation Loop
4. Coverslip
5. Microscope
6. Bunsen burner/sprit lamp
7. Young broth culture of motile bacteria

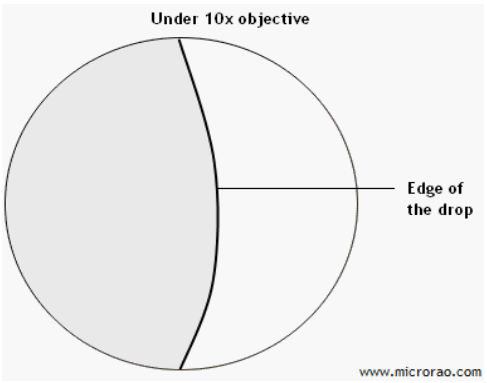
Procedure:

1. Take a clean glass cavity slide .
2. Hold a clean coverslip by its edges and carefully dab Vaseline on its corners using a toothpick.
3. Place a loopful of the broth culture to be tested in the center of the prepared coverslip.
4. Turn the prepared glass slide or concavity slide upside down over the drop on the coverslip so that the vaseline seals the coverslip to the slide around the concavity.
5. Turn the slide over so the coverslip is on top and the drop can be observed hanging from the coverslip over the concavity.
6. The drop is then observed under the low power (10x) dry objective of the compound microscope. The edge of the drop must be focused. Bacteria tend to accumulate at the edge of the drop.
7. Once the edge is located, it is then observed under 40x high power objective.



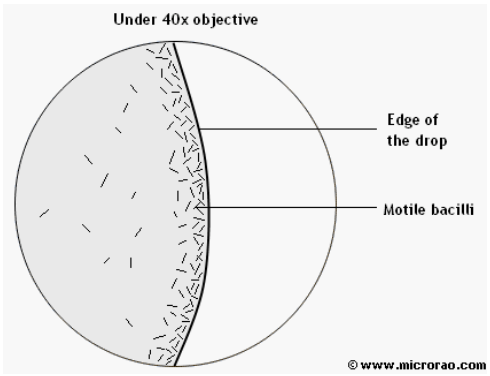


Observation under 10x objective:



Edge of the drop is seen.

Observation under 40x objective:



Motile bacilli are seen at the edge of the drop.

SIMPLE STAINING

Introduction

- Simple staining is a method of staining in which bacteria are stained by using a single stain.
- Simple staining is also called as monochrome staining or positive staining.
- Any basic dye such as methylene blue, safranin, or crystal violet can be used to color the bacterial cells
- In simple staining procedure cell are uniformly stained.
- The simple stain can be used as a quick and easy way to determine cell shape, size and arrangements of bacteria.

Principle :

These stains will readily give up a hydroxide ion or accept a hydrogen ion, which leaves the stain positively charged. Since the surface of most bacterial cells and cytoplasm is negatively charged, these positively charged stains adhere readily to the cell surface. After staining, bacterial morphology (shape and arrangements) can be appreciated.

Materials required:

1. Glass slide
2. Innoculation Loop
3. Microscope
4. Bunsen burner/sprit lamp
5. Young broth culture of motile bacteria
6. Stain

Procedure of simple staining

1. A clean grease free slide is taken .
2. On these grease free slide smear is made by using a sterile wireloop and cell suspension.
3. These slide is allowed to air dry .
4. After air drying these slide is rapidly passed through a flame for three to four times for heat fixation.
5. After heat fixation the slide is placed on the staining rack and flooded with a particular stain and these stain is allowed to react for three minutes.
6. Futher the slide is washed under running water.
7. The slide is air dried and washed under oil immersion.

Results

The bacterial cells usually stain uniformly and the color of the cell depends on the type of dye used. If methyene blue is used, some granules in the interior of the cells of some

bacteria may appear more deeply stained than the rest of the cell, which is due to presence of different chemical substances.

Application

- Simple staining procedure stain bacteria easily and helps in observation under microscope.
- It is useful in preliminary studies of morphological characters of cell that is its size, shape and arrangement.
- To differentiate bacteria from yeast cells