Ministry of Higher Education
and Scientific Research
University of Ishik
College of education
Department of Biology

Practical microbiology

Microscopic Identification of Bacteria

lab.6

2016- 2017 (3rd Grade)

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Identification of Bacteria

depend on:

1 – **Microscopic appearance** :-under the compound light microscope which include

a . Reaction with gram stain .

b .Morphology and arrangement .

c . Capsulated or not .

d . Motile or not .

e . Spore forming or not .

2 – **Macroscopic appearance** :include

a . Culture appearance .

b . Biochemical tests .

c . Gene tests .

There are **two** principal ways of preparing a microbial specimen for observation with light microscope:

1. Unstained smears (wet preparation):

To examine the **motility** of the bacteria.

2. Stained smears:

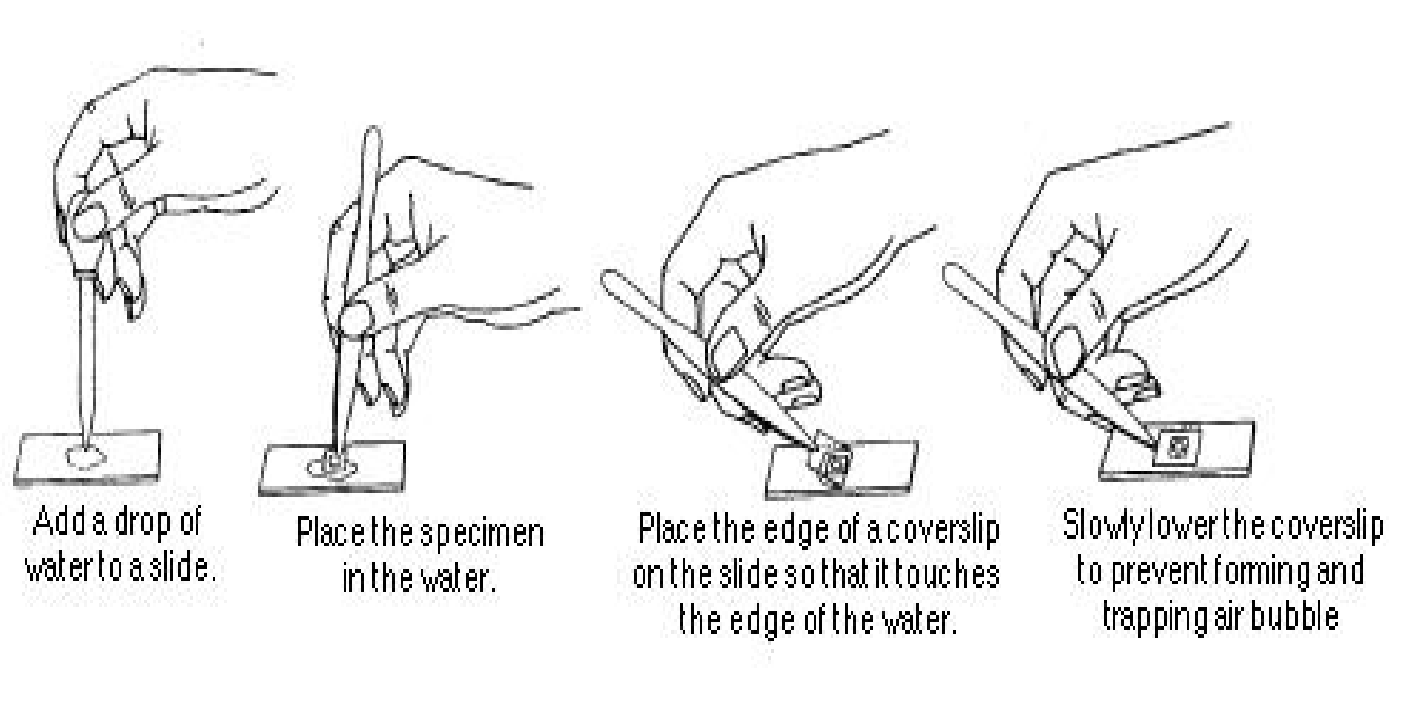
To study the **size, shape, arrangement** and staining affinity of the bacteria.

What is wet mount technique?

In a **wet mount**, the specimen is placed in a drop of water or other liquid held between the slide and the cover slip by surface tension. This method is commonly used, for example, to view microscopic organisms that grow in pond water or other liquid media, especially when studying their movement and behavior.

One problem for beginners is the difficulty of being able to see the organisms on the slide. Since bacteria are generally colorless and very transparent, the novice has to learn how to bring them into focus.

THE WET MOUNT SLIDE PREPARATION

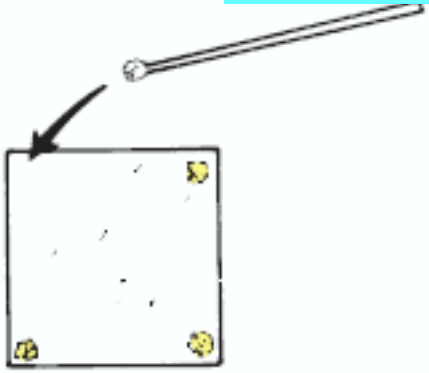


HANGING DROP AND WET MOUNT PREPARATIONS

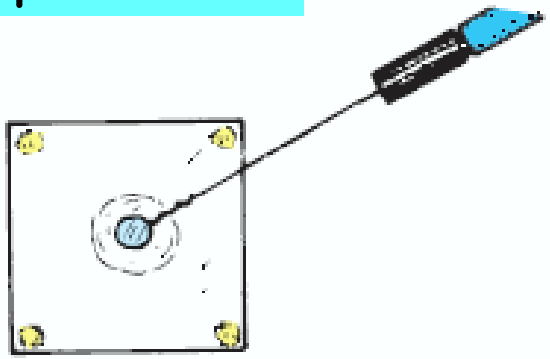
Hanging Drop Method Preparation.

Hanging drop preparation is a special type of wet mount (in which a **drop** of medium containing the organisms is placed on a microscope slide), often is used in dark illumination to observe the motility of bacteria

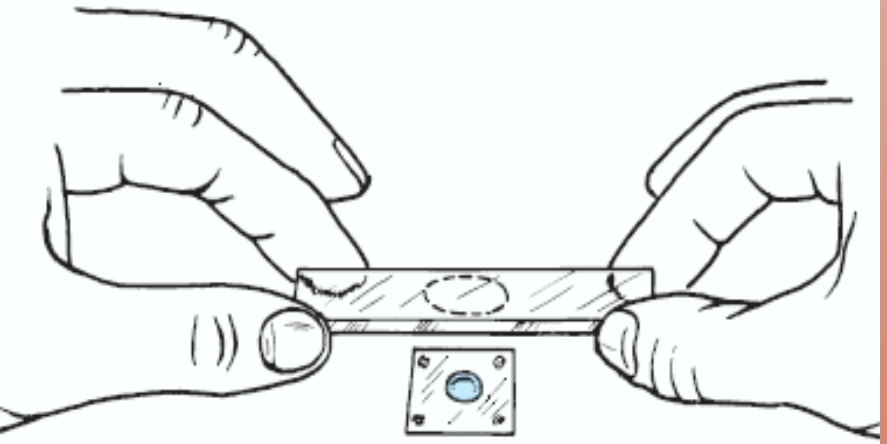
Hanging-drop slide preparation



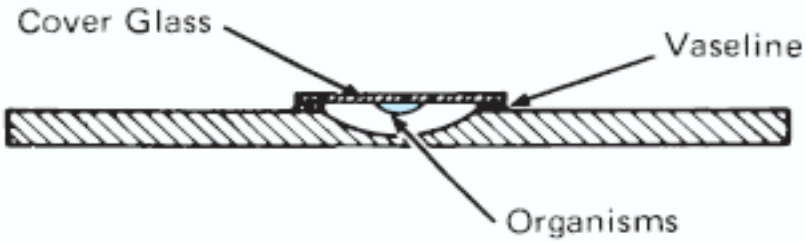
1 A small amount of Vaseline is placed near each corner of the cover glass with a toothpick.



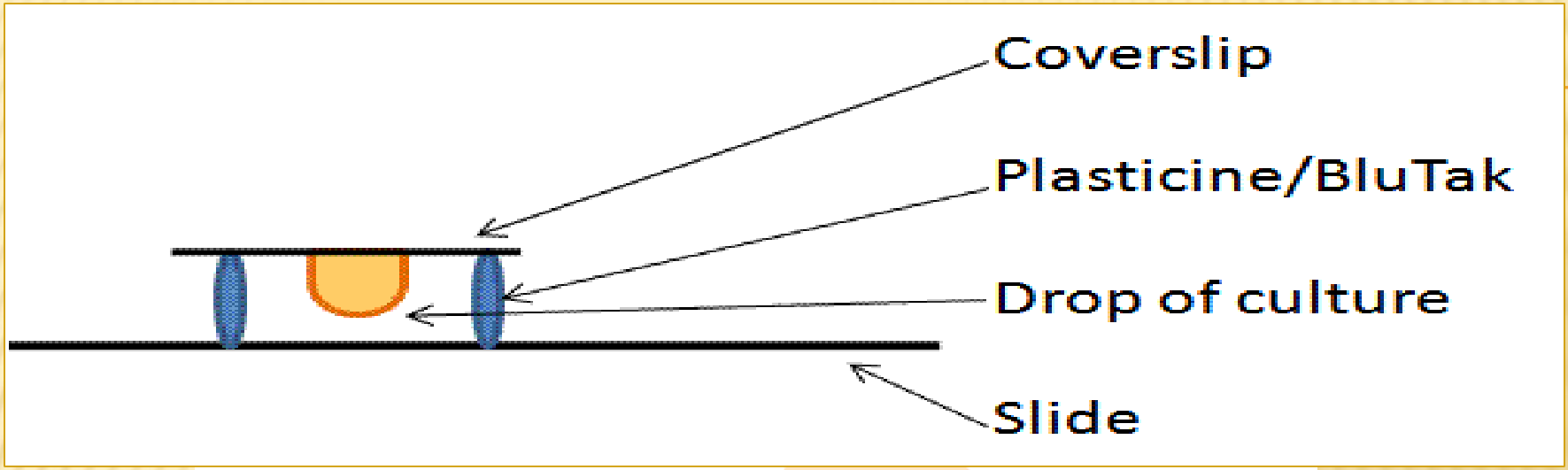
2 Two loopfuls of organisms are placed in center of cover glass.

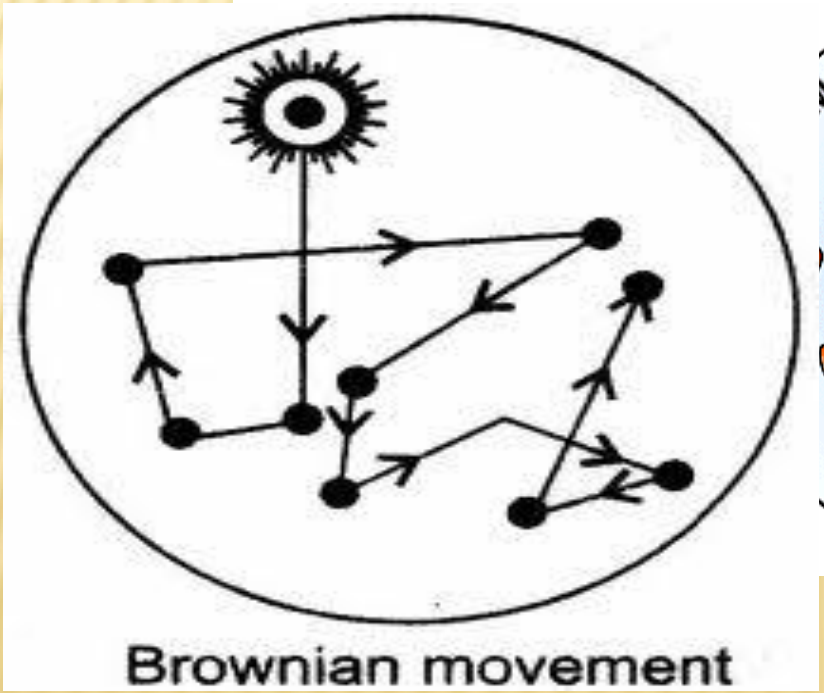


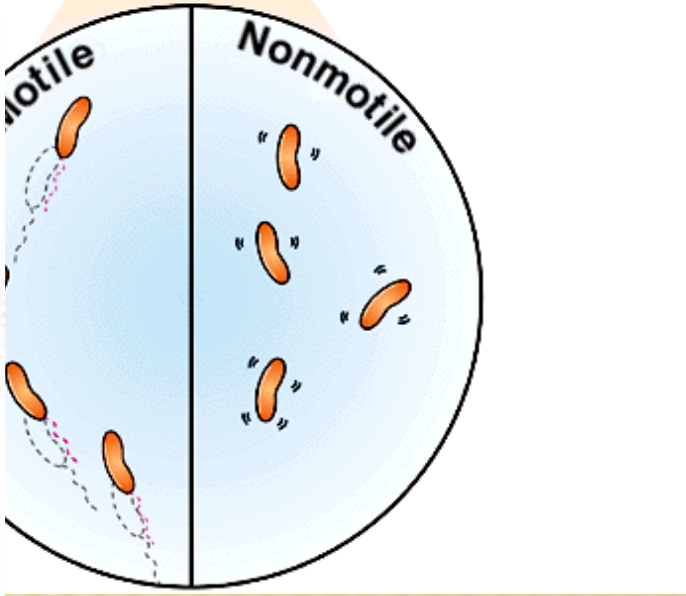
3 Depression slide is pressed against Vaseline on cover glass and quickly inverted.

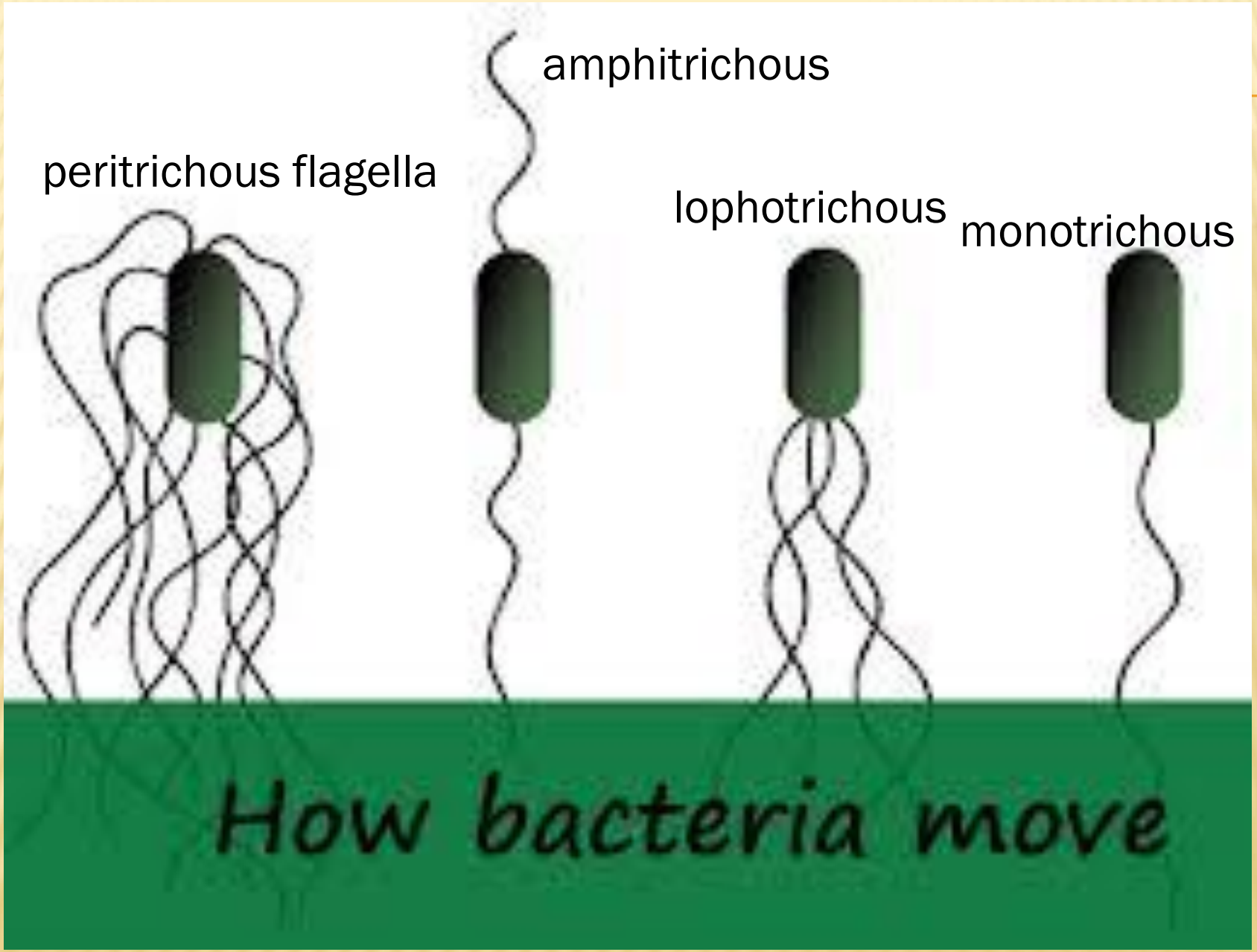


4 The completed preparation can be examined under oil immersion.


Coverslip
Plasticine/BluTak
Drop of culture
Slide

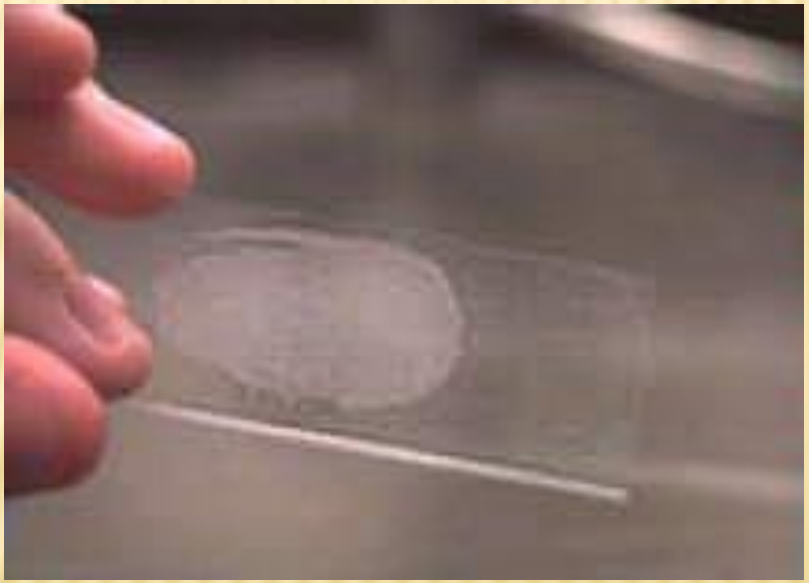

Brownian movement


Nonmotile


amphitrichous
peritrichous flagella
lophotrichous
monotrichous
How bacteria move

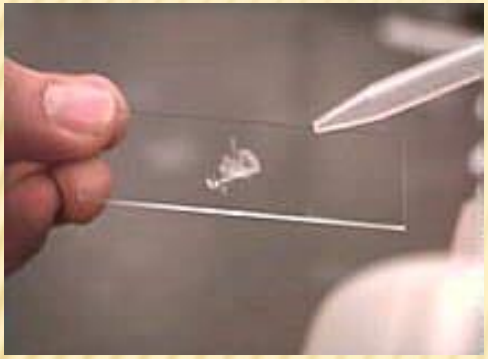
Smear preparation

The purpose of making a **smear** is to fix the bacteria onto the slide and to prevent the sample from being lost during a staining procedure. A **smear** can be **prepared** from a solid or broth medium

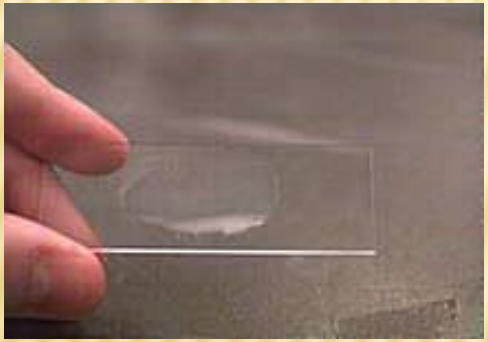


SMEAR PREPARATION

1. Place one needle of solid bacterial growth or two loops of liquid bacterial growth in the center of a clean slide.



2. If working from a solid medium, add one drop (and only one drop)
of water to your specimen with a water bottle. If using a broth medium, do not add the water



3-Now, with your inoculating loop, mix the specimen with the water
completely and spread the mixture out to cover about half of the total slide area

4. Place the slide on a slide warmer and wait for it to dry. The smear is now ready for the staining procedure.

Types of staining techniques

Simple staining
(use of a single stain)

For visualization of morphological shape & arrangement.

Differential staining
(use of two contrasting stains separated by a decolorizing agent)

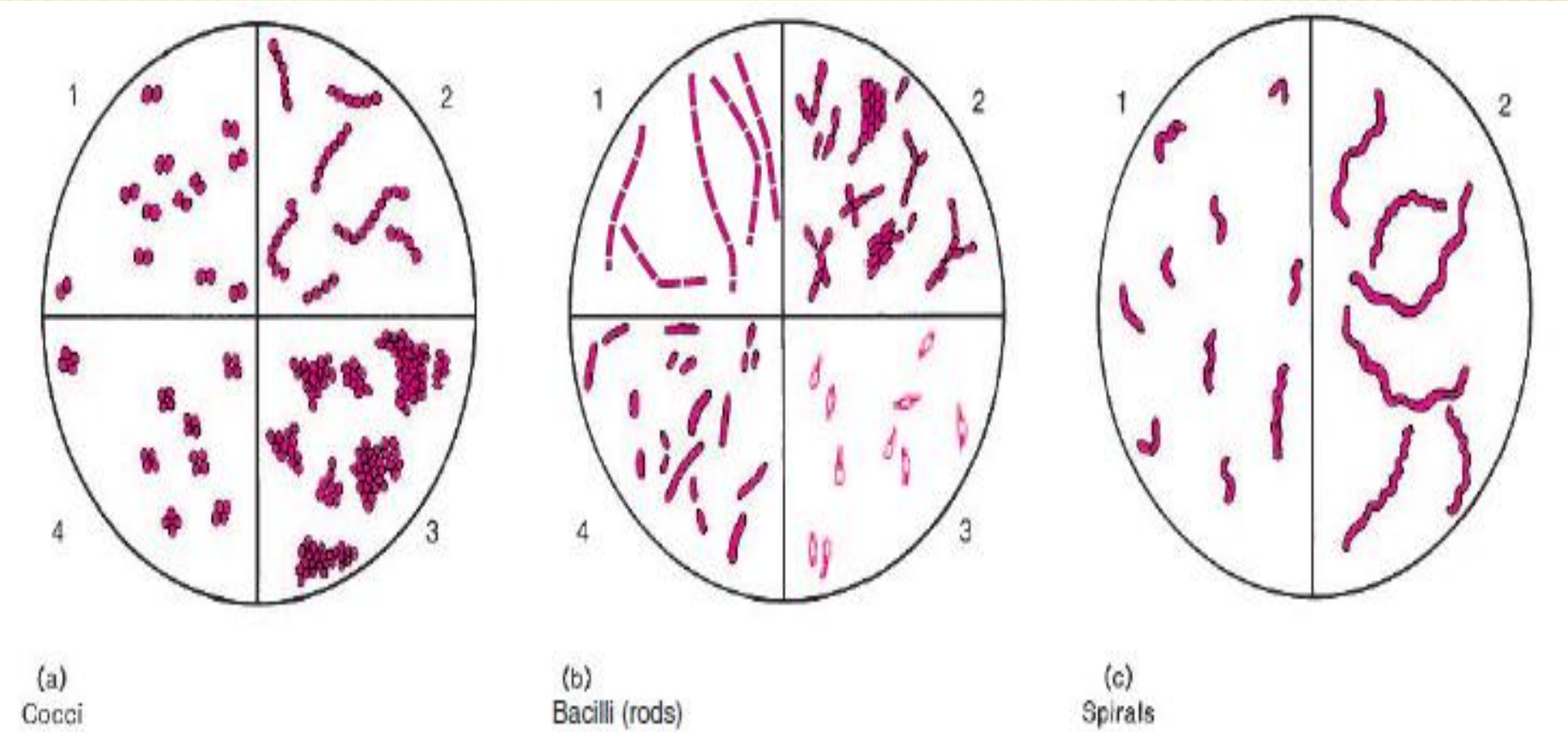
- Identification
  - Gram stain
  - Acid fast stain
- Visualization of structure
  - Spore stain
  - Capsule stain

SIMPLE STAIN

The use of a single stain to color a bacterial organism is commonly referred to as ***simple staining***. Some of the most commonly used dyes for simple staining are **methylene blue, basic fuchsin, and crystal violet**. All of these dyes work well on bacteria because they have color-bearing ions (chromophores) that are positively charged (cationic).

Simple staining is often employed when information about cell **size, shape,** and **arrangement** or grouping is required.

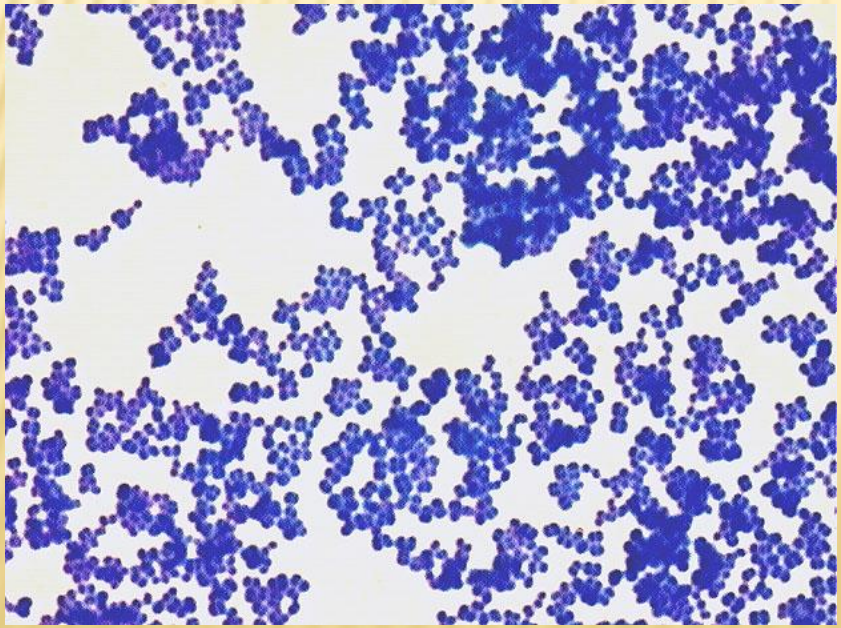
BASIC SHAPES AND ARRANGEMENTS OF BACTERIA

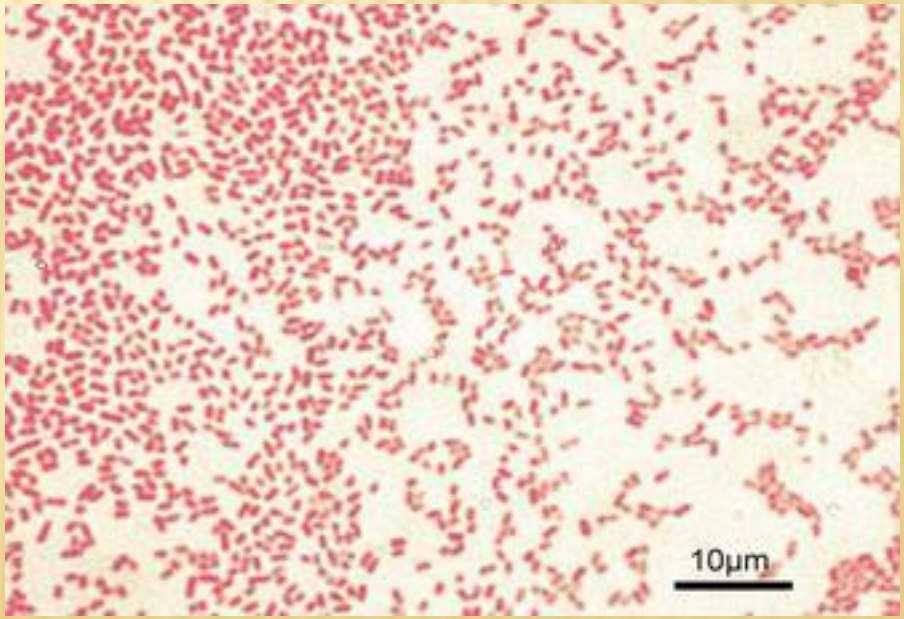


There are two types of stain for bacterial staining

1. Basic stains:

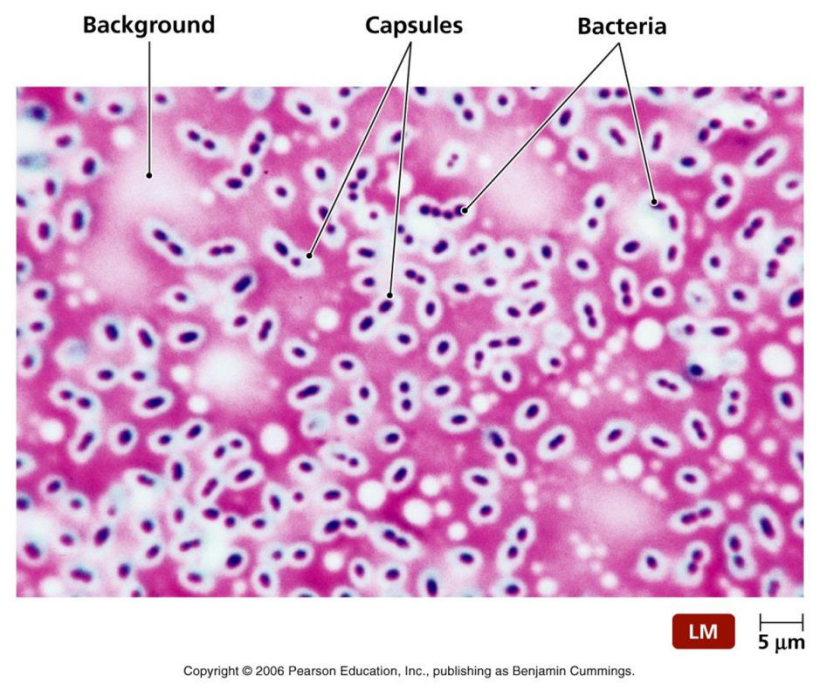
Basic stains carry positive charge which color bacterial cells because they are attracted to the negatively charged cell surface (bacteria). Basic stains include crystal violet, methylene blue, and safranin.

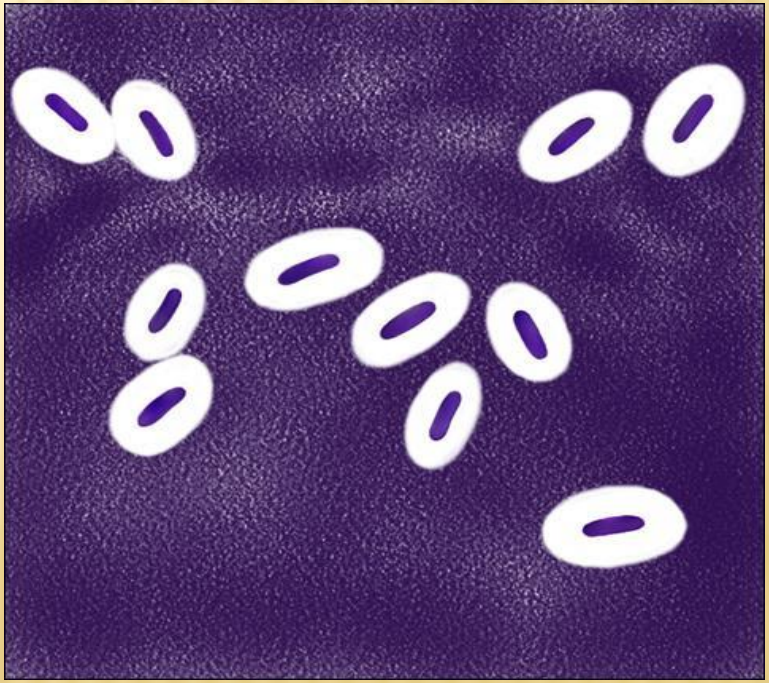




2. Acidic stain:

Acidic Stains carry negative charge that color the background surrounding bacterial cells. Acidic stains are repelled by the negatively charged bacterial cell surface and, hence, color only the background. Acidic stains include congo red, nigrosin, and india ink





THANK YOU FOR YOUR ATTENTION

- ANY QUESTIONS ??????