

## Simple stain

Bacteria are microscopic organisms. They are also colorless for the most part. In order to visualize them to study their structure, shape and other structural characteristics, it becomes necessary to make them more easily visible.

Stains or dyes are chemicals with two parts – the part that has the color is called the **chromophore** and the other part that helps in the coloring properties is called the **auxochrome**.

The chromophore of a stain may be charged –either positive charge or negative charge. A stain with a positively charged chromophore is called a **cationic dye** and one that has a negatively charged chromophore an **anionic dye**.

These are also classified based on the conditions in which they stain the best.

Staining procedure can also be classified in different ways.

**Simple** staining involves the use of only 1 dye and is used primarily as a means to study the morphology and structure of organisms.

**Differential** staining uses more than 2 dyes and is also used to differentiate the organisms into one of two groups.

**Simple staining** – there are two methods:

**positive staining** - where the actual cells are themselves colored and appear in a clear background;

**negative staining** – where the cells remain clear (uncolored) and the background is colored to create a contrast to aid in the better visualization of the image.

Negative staining is **preferable** since it uses chemical fixation rather than heat fixation and thus causes less distortion of the cells.

### Procedure:

1. prepare smear and fixation.
2. Add stain like crystal violet 10sec. ,methylene blue and safranin 30-63 sec. and Pour excess dye .
3. washing the smear in tap water.
4. dry the water in slide and examine under oil emersion and write result .

## Gram's stain :

**Gram staining** (or **Gram's method**) is a method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative). The name comes from its inventor, Hans Christian Gram.

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in a thick layer in Gram-positive bacteria. A Gram-positive results in a purple-blue color while a Gram-negative results in a pink-red color.

### REAGENTS USED IN GRAM STAIN :

1. Gram Crystal Violet 0.5%
2. Gram Iodine
3. Gram Decolorizer
  - a. Methanol 80%
  - b. Acetone 20%

## Gram Stain Procedure

1. Flood the Smear with **Crystal Violet** Allow to stand for 1 min Rinse with water to remove excess stain .
2. Flood the Smear with **Iodine** solution Allow to stand 2 min Rinse with water to remove excess Iodine.
3. Drip Decolorizer (80% Methanol +20% Acetone) across the slide about 5 sec the effluent should appear pale or clear Rinse with water to remove excess alcohol .
- 4 Flood the slide with **Safranin** solution let stand for 2 minutes Rinse with water to remove excess stain blot dry
- 5.Observe under Oil Immersion .

## Acid fast stain

The **Ziehl–Neelsen stain**, also known as the **acid-fast stain**, was first described by two German doctors: the bacteriologist Franz Ziehl (1859–1926) and the pathologist Friedrich Neelsen (1854–1898). It is a special bacteriological stain used to identify acid-fast organisms, mainly Mycobacteria. ***Mycobacterium tuberculosis*** is the most important of this group because it is responsible for tuberculosis (TB). Other important Mycobacterium species involved in human disease are ***Mycobacterium leprae***, ***Mycobacterium kansasii***, ***Mycobacterium marinum***, and members of the *Mycobacterium avium* complex. Acid fast organisms like Mycobacterium contain large amounts of lipid substances within their cell walls called mycolic acids. These acids resist staining by ordinary methods such as a Gram stain. It can also be used to stain a few other bacteria, such as *Nocardia*. The reagents used are Ziehl–Neelsen carbol fuchsin, acid alcohol, and methylene blue. Acid-fast bacilli will be bright red after staining.

## :Procedure

1. Drop suspension onto slide
2. Air dry slide 10 minutes at 60 °C, heat-fix slide 10 minutes at 90 °C
3. Flood slide with Carbol Fuchsin
4. Hold a flame beneath the slide until steam appears but do not allow it to boil
5. Allow hot slide to sit for 3 to 5 minutes, rinse with tap water
6. Flood slide with 3% hydrochloric acid in isopropyl alcohol
7. Allow to sit 1 minute, rinse with tap water
8. Flood slide with Methylene Blue
9. Allow to sit 1 minute, rinse with tap water
10. Blot dry
11. View under oil immersion lens

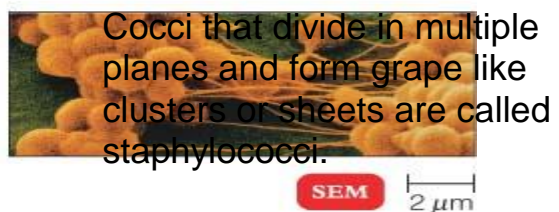
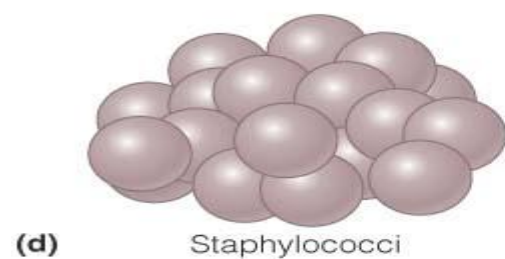
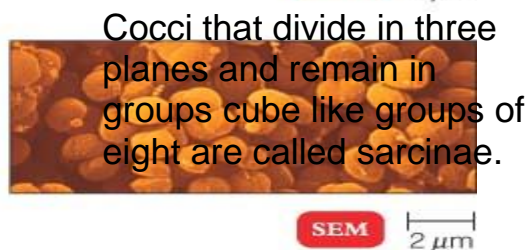
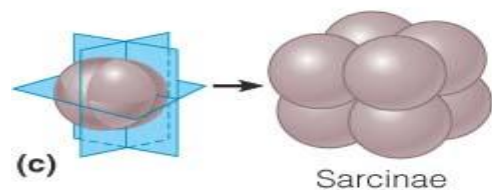
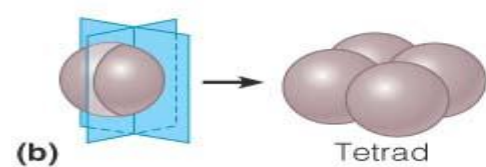
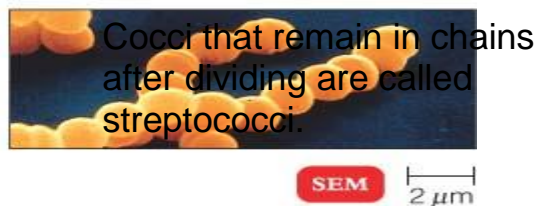
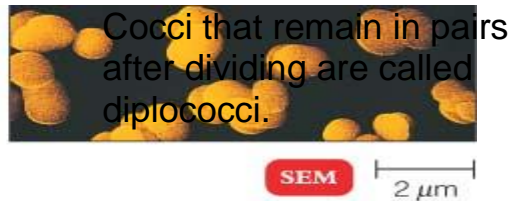
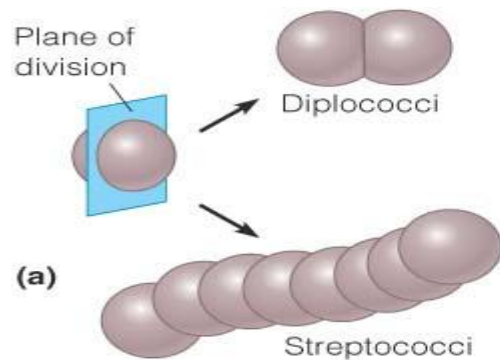
## Bacterial shapes

The three basic bacterial shapes are coccus (spherical), bacillus (rod-shaped), and spiral (twisted), however pleomorphic bacteria can assume several shapes.

## Arrangement of cocci

Cocci may be oval, elongated, or flattened on one side.

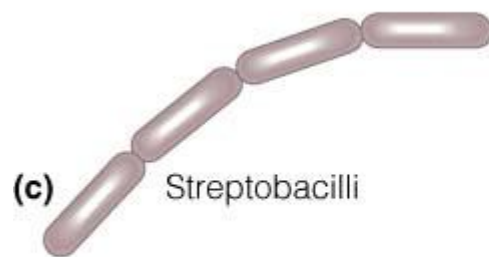
Cocci may remain attached after cell division. These group characteristics are often used to help identify certain cocci.



# Bacilli

Since bacilli only divide across their short axis there are fewer groupings.

Bacillus is a shape (rod shaped) but there is also a genus of bacteria with the name *Bacillus*. You wouldn't confuse the two, since you know the rules for writing the genus and species names of organisms, right.



SEM 2  $\mu\text{m}$



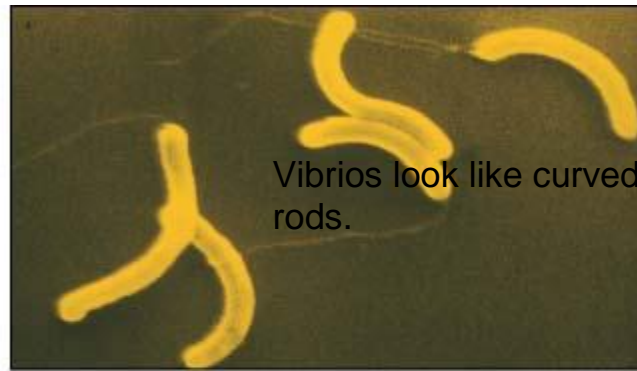
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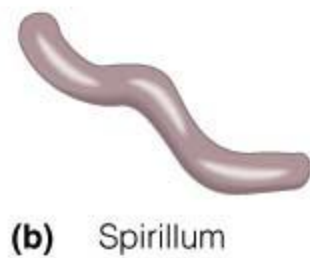
SEM 1  $\mu\text{m}$

# Spiral bacteria

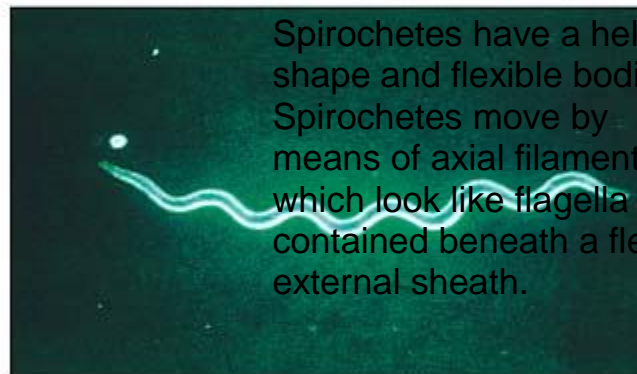
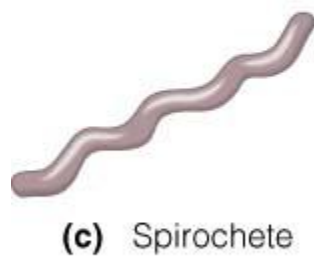
Spiral bacteria have one or more twists.



SEM 2  $\mu$ m



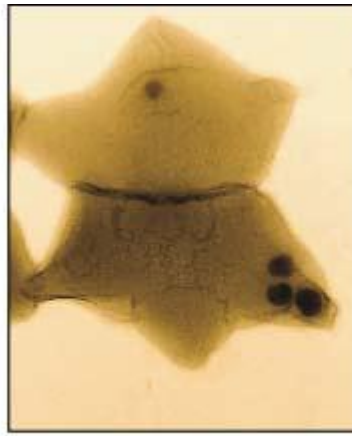
SEM 2  $\mu$ m



SEM 5  $\mu$ m



**(a)** Star-shaped bacteria

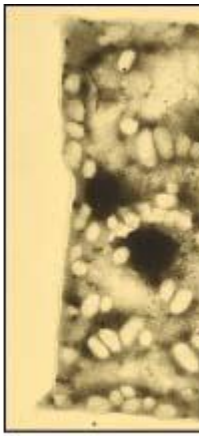


LM

0.5  $\mu\text{m}$



**(b)** Rectangular bacteria



LM