Staining of bacteria

- Microorganisms are transparent
- So, it is difficult to see them in an unstained state.
- When stained, they become opaque and the contrast between them & the surrounding medium is increased.
- they become clearly visible.

Importance of staining:

- To describe morphological features.
- To preserve them for future study.
- To differentiate bacteria.

Preparation of smear:

- Smears should be spread evenly covering an area of about 15-20mm diameter on a slide.
- Purulent material
- Non purulent material
- Sputum
- Swab
- Culture
- Labelling of smear :
- Every slide should be labelled clearly with numbers or letters by diamond or grease pencil.

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- Fixation of smear: It is a process by which the internal and external structures of the cells and microorganisms are preserved & fixed in position. It also prevent smear being washed from slides during staining procedure. it also kills the microbe.

- There are two types (methods) of fixation:

Physical Method: Heat fixation

- Allow the smear to air-dry completely.
- Rapidly pass the slide with smear upper most, three times through the blue flame of the spirit lamp or pilot flame of Bunsen burner. Allow the smear to cool before staining.
- Disadvantage: It can damage the organism & alter the staining reactions especially when excessive heat is used.
- It also damages leucocytes. So it is unsuitable for fixing smear which contains intracellular organisms such as N.gonorrhoeae & N.meningitidis.

Chemical fixation:

- Chemicals - Alcohol , Acetic acid, Mercury chloride , Formaldehyde , Glutaraldehyde
- Method:

1. Allow the smear to air-dry completely.
2. Add one or two drops of chemicals.
3. Leave the alcohol on smear for a minimum 2 min, or until the chemical dries on the smear.
4. Advantage: Less damaging to microorganism than heat.
5. Pus cells are well preserved. So recommended for fixing the smear when looking for gram negative intracellular diplococci.

- It is more bactericidal than heat. So it is preferable to fix the smear prepared from sputum (M.tuberculosis)

Types of stain:

1) Simple stain:

- Single staining agent is used
- Basic dyes such as crystal violet, methylene blue, carbol fuchsin are used.

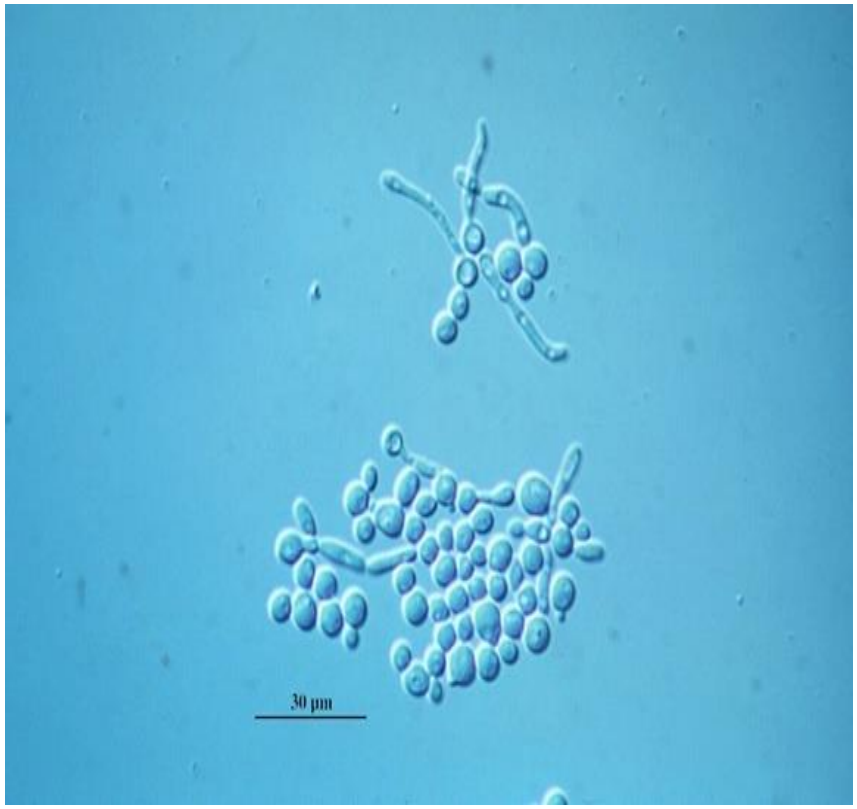
2) **Differential stain:**

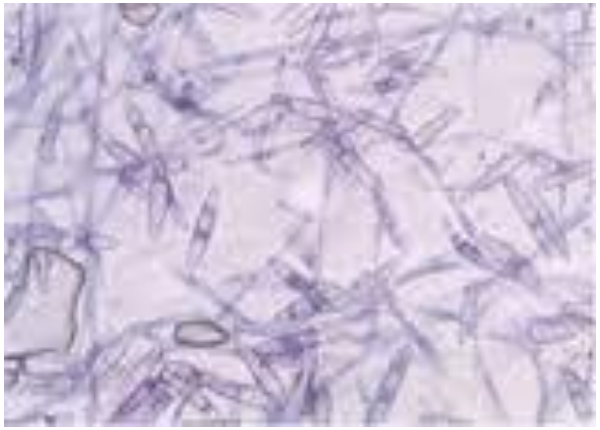
- Divide bacteria into separate groups based on staining properties. Gram stain, Acid fast stain

3) **Stains used for specific structures**

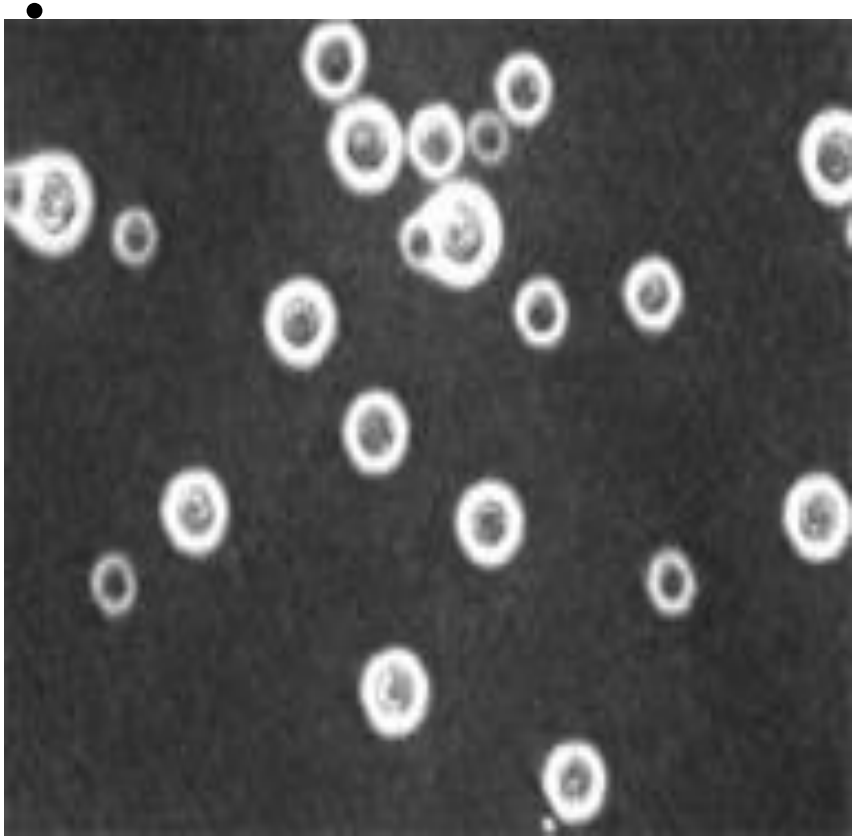
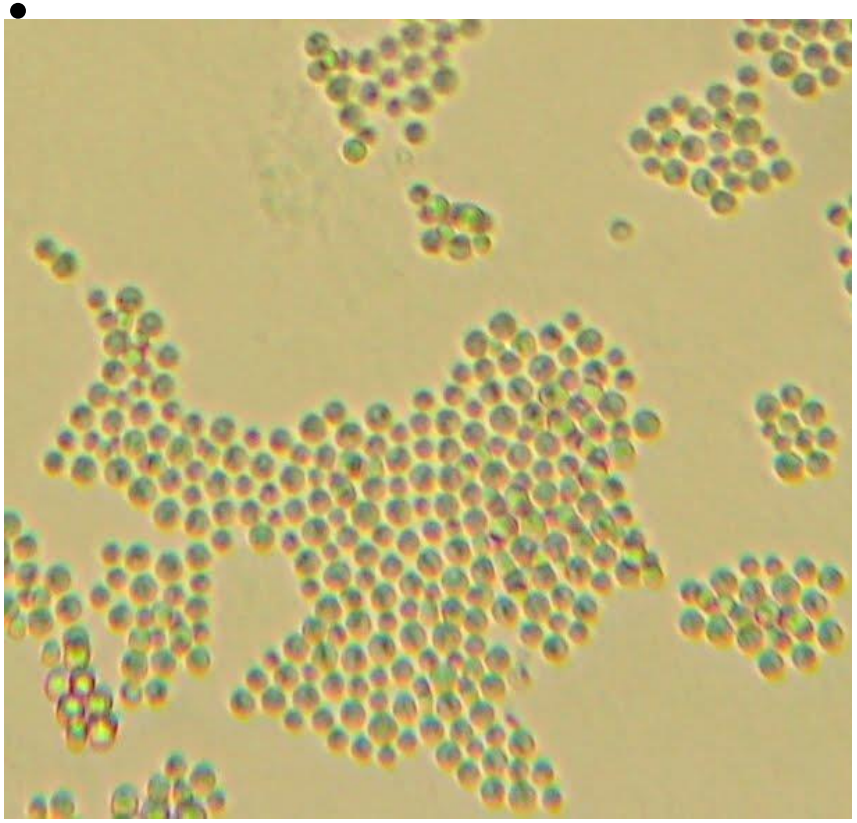
- Negative staining - for capsule demonstration dye such as India ink or nigrosin used
- Impregnation stain - Bacterial flagella are thin, thread like structures which cannot be seen under light microscope. They are thickened by coating them to see under light microscope.
- Modified acid fast stain — for spore demonstration
- Albert stain — for metachromatic granules

SIMPLE STAIN

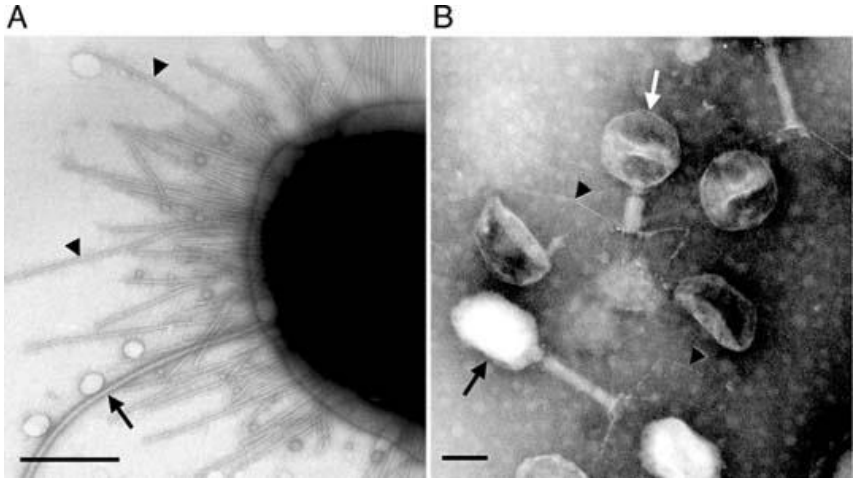




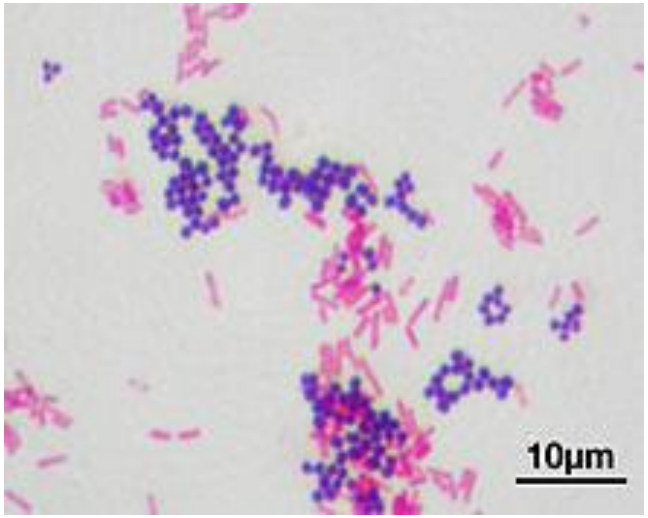
NEGATIVE STAIN

- 
- 

IMPREGNATION STAIN



DIFFERENTIAL STAIN



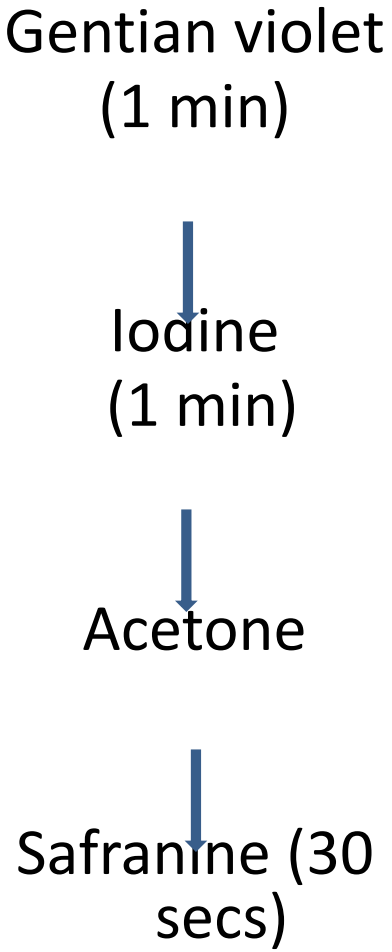
GRAM STAIN

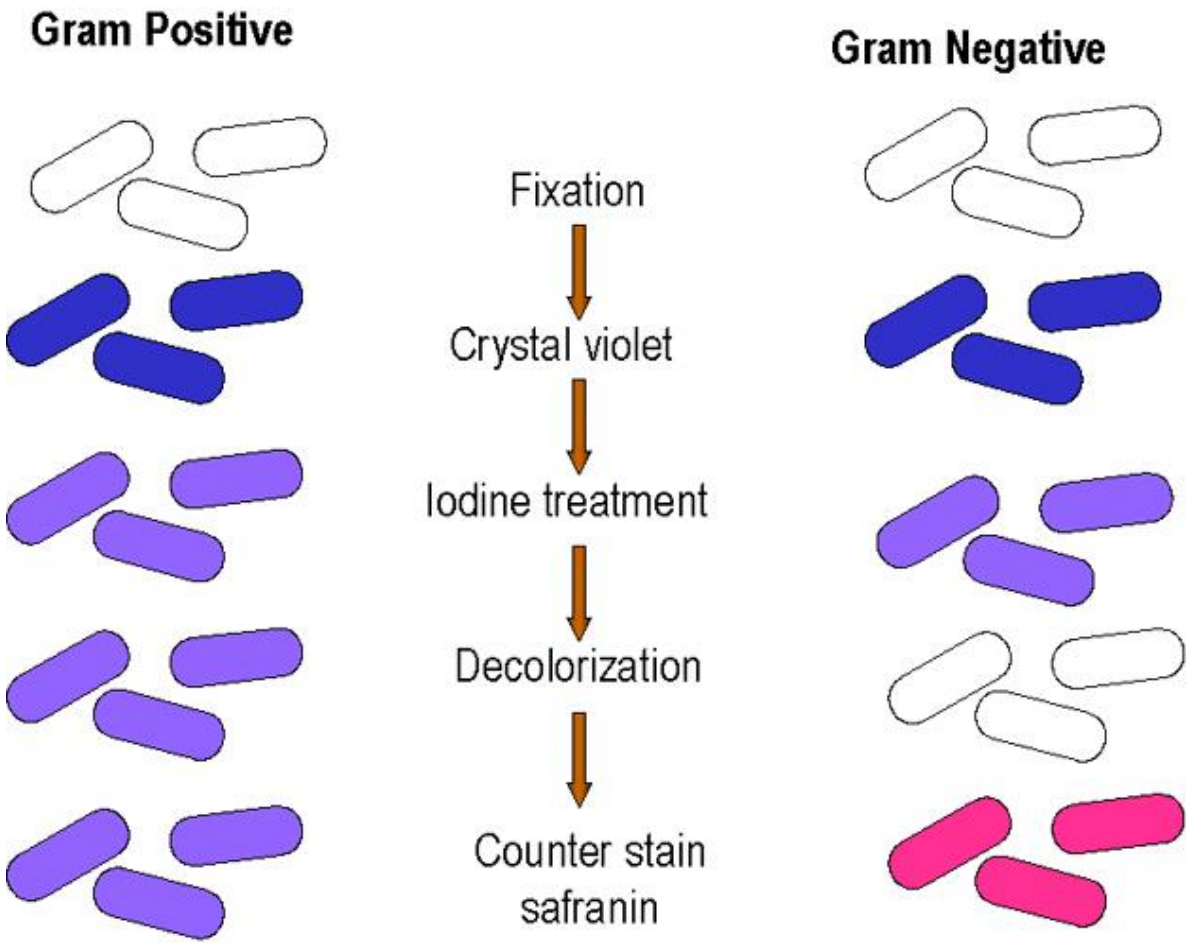
- Developed by the Danish physician Christian Gram in 1884.
- Most widely employed staining method in bacteriology.
- It is a differential staining procedure because it divides bacteria into two classes - Gram positive & Gram negative.
- **Principle:**
- Crystal violet (gentian violet) serves as the primary stain, binding to the bacterial cell wall after treatment with a weak solution of iodine which serves as the mordant for binding the dye.
- Some bacterial species, because of the chemical nature of their cell wall, have ability to retain the dye iodine complex appear violet even after treatment with an organic decolorizer ethanol or acetone.
- Dye retaining bacteria appear purple (gram positive) while which are decolorized take the counter stain (safranin) and appear pink.(gram negative).

Theories of Gram stain:

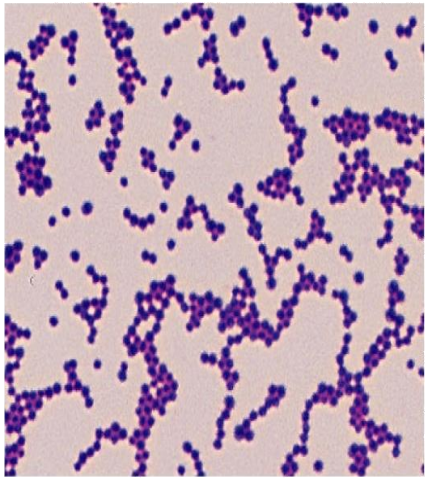
- Lipid content theory
- Magnesium ribonuclease theory
- Iso electric ph theory

- **Procedure:**
- Lay the fixed slide on a staining rack cover the smear completely with the gentian violet. Keep it for 1 minute.
- Pour off the stain and flood the slide with Gram's iodine solution and wait for 1 minute.
- Gently drain off the iodine solution and rinse with running tap water.
- Decolorize rapidly with acetone or ethanol by holding the smear between thumb and forefinger.
- Wash the slide under running tap water and drain.
- Pour safranin solution on the slide and keep it for 30 seconds.
- Wash off the stain with tap water.
- Wipe the back of the slide clean and place in a draining rack for the smear to air-dry. Examine the smear microscopically with oil immersion objectives.




Gram Positive
Gram Negative
Fixation
Crystal violet
Iodine treatment
Decolorization
Counter stain
safranin

- **Results:**
- Gram positive bacteria - dark purple
- Yeast cells - dark purple
- Gram negative bacteria - pale to dark pink
- Nuclei of pus cells - red
- Epithelial cells — pale red



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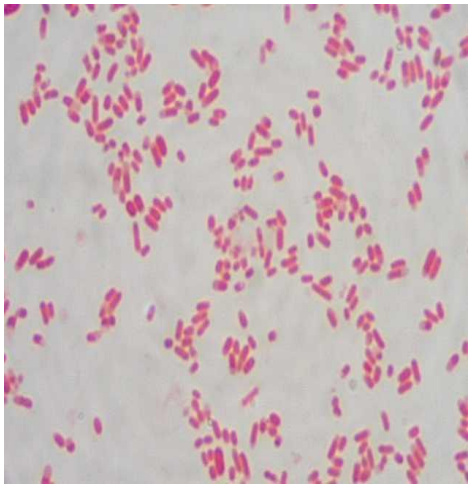

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- **Substitute for primary stain**
- Methyl violet,
- Crystal violet
- **Substitute for secondary stain**
- Carbolfuschin
- Phenol red
- Basic fuschin
- **Substitute for Decolorizer**
- Alcohol

THANK YOU