Staining of bacteria

Stains are employed in bacteriology for making organisms visible.

Purpose of staining:

1- to explain the morphology & arrangement of bacteria.

2-to explain some structure of bacteria such as capsule or flagella.

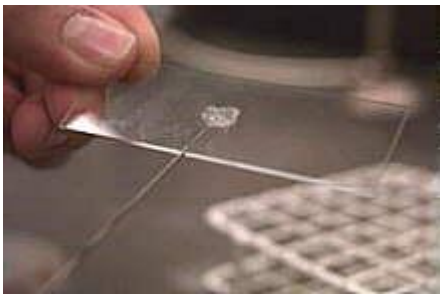
3-differantiated of bacteria according to cell wall.

which can be divided into three groups:

- ❖ Simple stains: methylene blue and safranin.
- ❖ Complex stains: gram stain and acid fast stain.
- ❖ Special stain: capsule S. and flagella S.

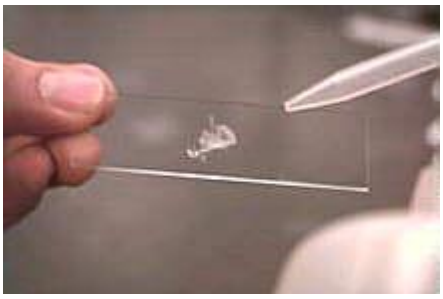
Smear preparation:The preparation of a smear is required for many laboratory procedures, including the Gram-stain. The purpose of making a smear is to fix the bacteria onto the slide and to prevent the sample from being lost during a staining procedure. A smear can be prepared from a solid or broth medium. Below are some guidelines for preparing a smear for a Gram-stain.

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

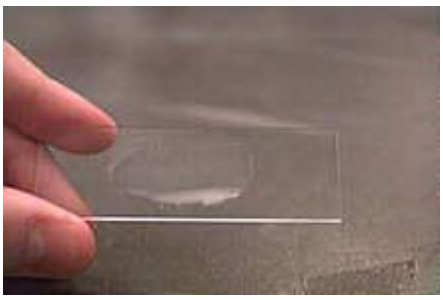


2. If working from a solid medium, add one drop (and only one drop)

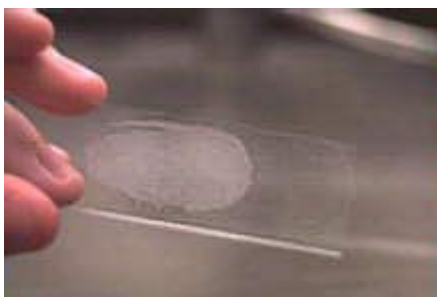
of water to your specimen with a water bottle. If using a broth medium, do not add the water.



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.



-Method of simple staining:

1-Flood the slide with methylene blue for 1 min.

2-Wash off the stain with slowly running tap water.

3-Allow the slide to dry in air or placed it between two sheets of filter paper (called blotting the slide).

4-Examine under oil immersion lens, see the shape and arrangement of the bacteria.

-Gram staining method:

1- Prepare the bacterial smear.

2- Flood the slide with crystal violet leave to all for 1-2 min. Wash with tap water.

3- Apply gram's iodine (lugol's iodine) . Leave to all one minute. Wash with tap water.

4- Apply 95% ethyl alcohol (a decolorizer). Leave to all for 20-30 seconds. Wash with tap water.

5- Apply diluted carbol – fuchsine (the counter stain) leave to all for 1 min. wash with tap water.

6- Blot, dry in air and examine with oil immersion lens.

it is possible to differentiate between bacteria of the same morphology.

Base of gram – stain

Gram +ve had thick layer of Peptidoglycan which give rigidity and strong integrity to the cell wall and not affected by alcohol. Also it contains tiechoic acid which are the source of mg+2.

Gram-ve head thin layer of Peptidoglycan with thick layer of lipid containing layer (lipoprotein, Lipopolysaccharid and phospholipids) and those it affect more by alcohol.

Gram + cell wall	**Gram _ cell wall**
Thick peptidoglycan	Thin peptidoglycan
Teichoic acids	No teichoic acids
Not many polysaccharides	Outer membrane has lipids polysaccharids L.P.S
Acid-fast cells	No acid fast cell

-Special stains: - stains for special morphological structures such as flagella, spores capsule.

Capsule: it is extracellular material surrounding bacterial cell wall; consist of polysaccharide i.e. (Strep. Pneummoniae) or polypeptides (E. coli and klebsiella) or polysaccharide and polypeptide (B. anthracis).

Its function:-

Its form as protective covering against phagocytosis.

Inhibit the killing factors in the serum of the host.

Capsule seen by:

1-) india ink method

2-) Hiss's stain

3-) Anthony,s capsule stain procedure

1.Prepare the bacterial smear and not fix to avoid destroying or distorting the capsule or causing shrinkage).

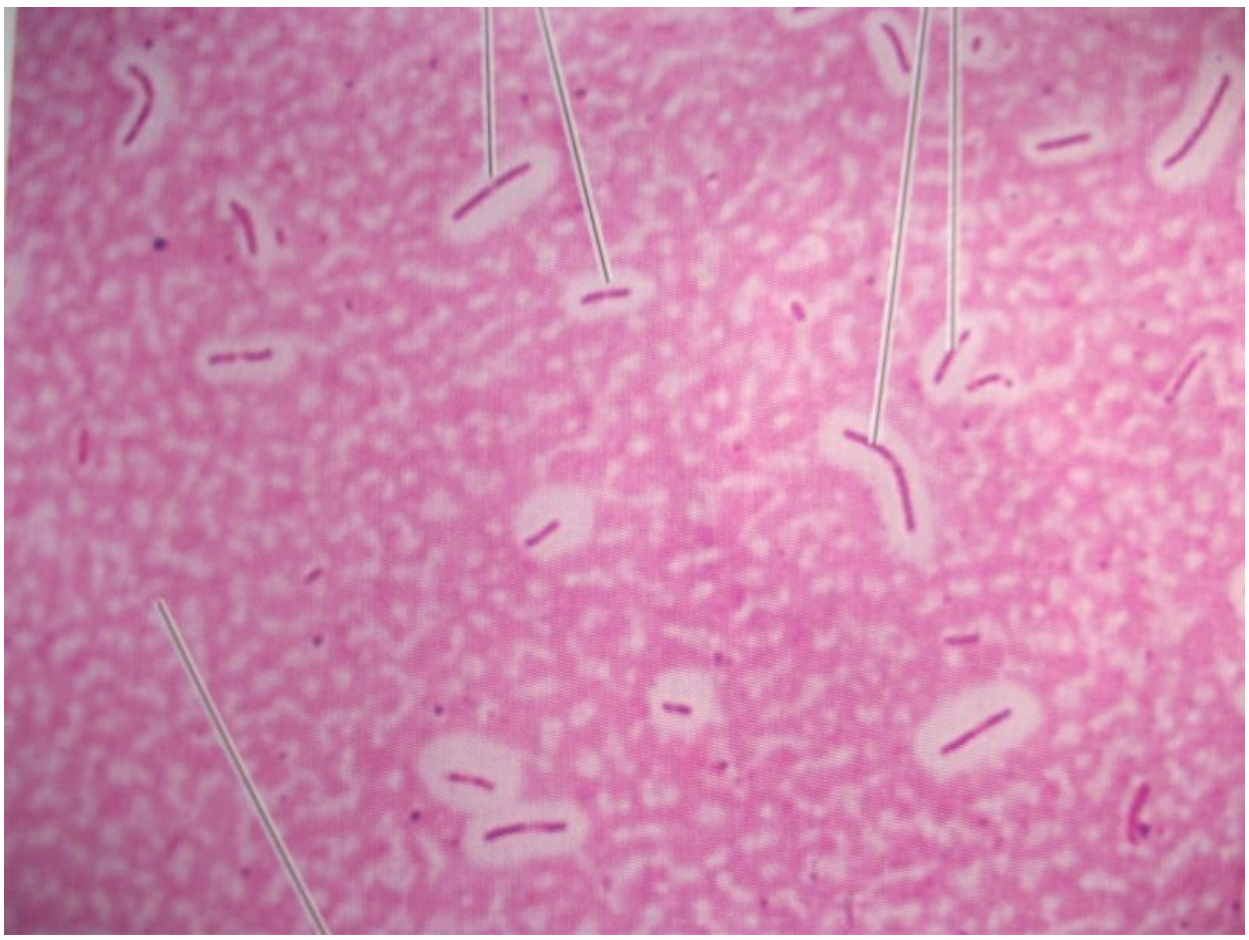
2.Flood the slide by crystal 1% for (2) minutes.

3.Wash gently with (decolorize) 20% copper sulfate for (20) sec.

4.Dry in air.

5.Examine under oil immersion.

The bacterial cells and the background will be stained by the crystal violet while the unstained capsule will appear transparent(white). 20% of copper sulfate serves as the mordant stabilize the capsule structure.



spore stain: it is a special stain for spores which are common in the aerobic genus Bacillus and the anaerobic genus Clostridium. Endospores differ in their shape and situation according to the type of bacteria:

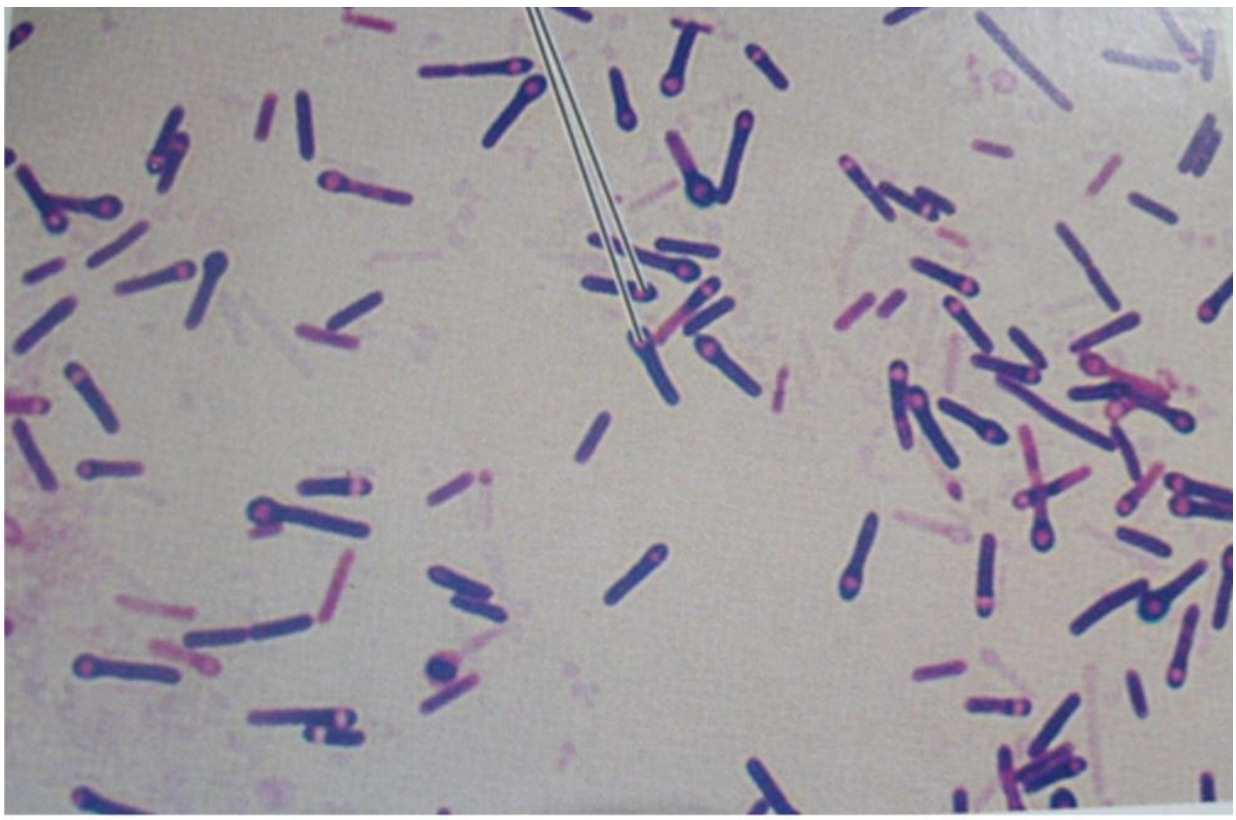
- Circular and central in *B. anthracis.*
- Circular and sub terminal in *Cl. chauvoei*.
- Circular and terminal in *Cl. tetani*.

 Oval and terminal in *Cl. tertium*.

Method of spore stain (modified Ziehl – Neelsen):

1. Prepare bacterial film (smear).
2. Flood the slide with malachite green, heat until steaming for5 min.
3. Cool the slide then wash with tap water.
4. Dipping the slide in 3% Acetic acid for 20-30 sec.
5. Wash with tap water.
6. Flood the slide with counter stain (methylene blue or malachite green) and leave for 1 min.
7. Wash with tap water.
8. Blot and dry in air.
9. Examine under oil immersion.

endospores colored green. Parent cells stain red.



Gram stain of the Gram-positive rod *Clostridium tetani*, the causative agent of tetanus.