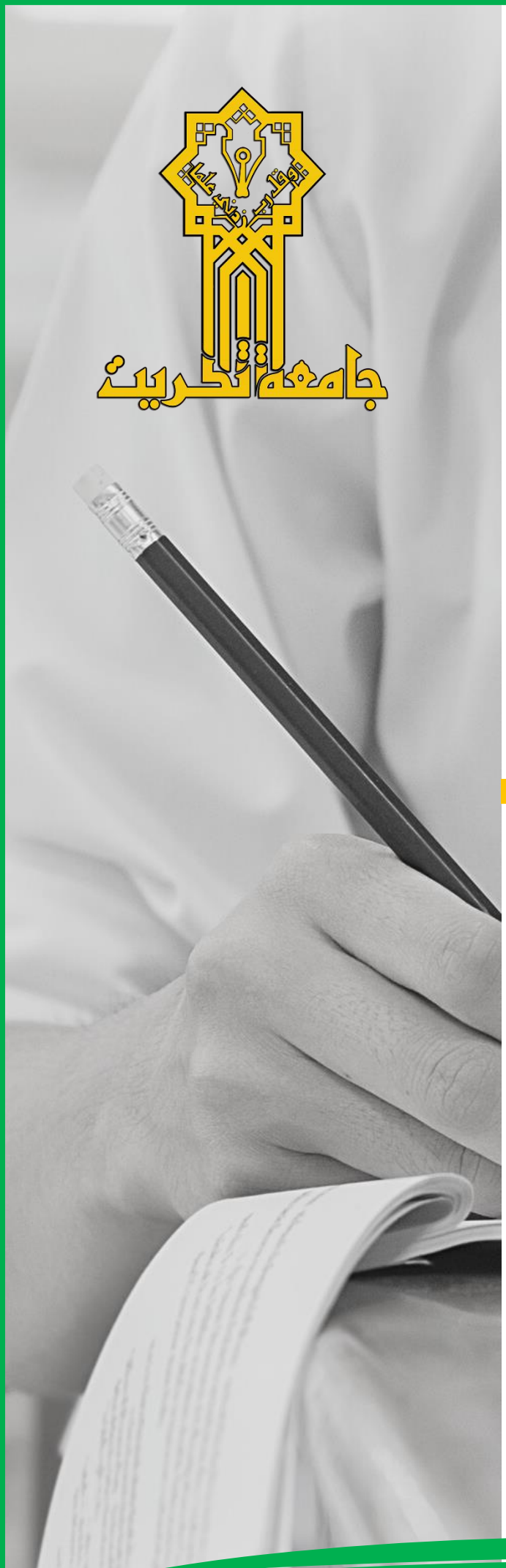

Tikrit University
College of Veterinary Medicine

Lect.6: Microbiology

Subject name: Bacterial staining

Subject year:Third-year

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SCAN ME
Lecturers link

Stains are:

1-Simple stains:

a-Basic dye. **b-**Acidic dye.

2-Differential stains:

a-Gram stain . b-Acid-fast stain.

3-Structural stains:

a-Feulgen stain. b-Endospore stain. c-Cell wall stain. d-Capsule stain. e-Flagella stain

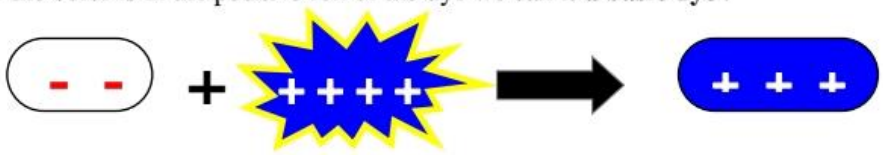
Dyes:- are generally salts in which one of the ions are colored. A salt is a compound composed of a positive and negative ions.

1-Simple stains: single stain to color the organism .

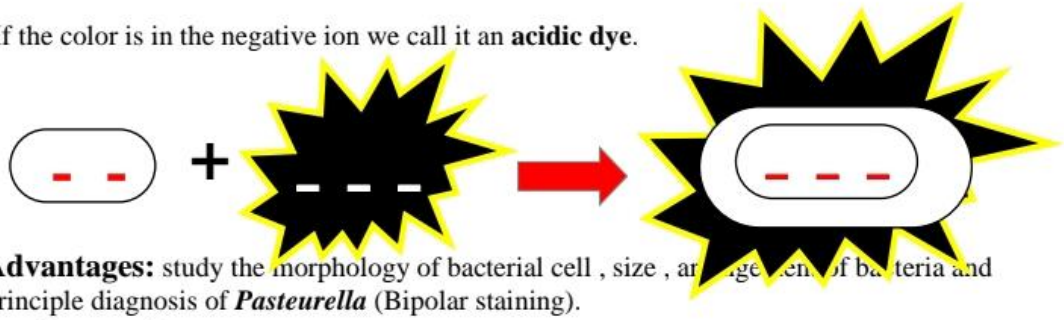
The simple dye methylene blue is the salt blue chloride:

MBCI → MB +Cl

If the color is in the positive ion of the dye we call it **a basic dye .**



If the color is in the negative ion we call it an **acidic dye**.



Advantages: study the morphology of bacterial cell , size , arrangement of bacteria and principle diagnosis of ***Pasteurella*** (Bipolar staining).

Smear Preparation:

1- Clean the slide with 50% of ethyl alcohol.

2- If the bacteria are growing in a liquid media, one starts by placing two loopful of liquid media directly on the slid. From solid media, one starts by placing one or two loopful of water on the slide and mix with one loopful of organism.

3- Spread the drop on the slide to form thin film.

4- Allow the slide to dry in the air.

5- When the film dry, pass the slide, film side up three times through the Bunsen flame.

6-Staining.

Crystal violet	Methylene blue
Safranin	Carbol fuchsin

The purpose of fixation:

is to kill the micro organisms cause it to adhere to the slide.

2-Differential stains:

a-Gram stain

It is divide bacteria in to two groups gram positive , gram negative. The difference in staining is due to the variance in surface layer of the two types of cells. Gram positive consist of thick layer of peptidoglycan which resist decolorize with alcohol so gram

positive bacteria retain a crystal violet through decolonization and appear purple, but gram negative consist of vary thin layer of peptidoglycan and lipopolysaccharide which decolorize with alcohol and take the counter stain with red dye of safranin.

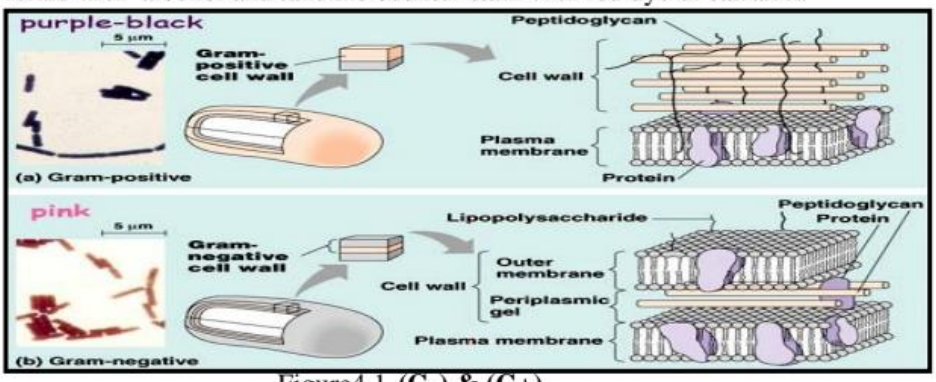


Figure4.1 **(G-) & (G+)**

Gram's stain steps

1-Prepare smear and fixed.

2-Crystal violate for 30 second and wash with water.

3-Gram s iodine for 30 second and wash with water.

4-Decolorized with alcohol 95% for 10 – 20 second and wash with water.

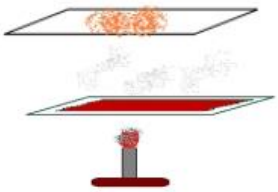
5-Counter stain (Safranin) for 30 second and wash with water and examine with oil immersion lens

b-Acid-fast stain(Ziehl-Neelsen stain).

Bacteria in the genus *Mycobacterium* and some in the genus *Nocardia* contain a waxy material in their cell wall called mycolic acid. If they are stained with carbol fuchsine and heat is applied during the staining procedure, the carbol fuchsine is able to penetrate the cell and it is not removed by subsequent washing with acid-alcohol. This method is important in diagnosis tuberculosis, and leprosy in human .

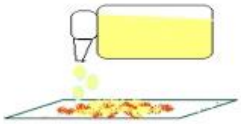
Staining Method

1-Prepare the smear of Mycobacterium sometime killed 40% formalin of sputum .

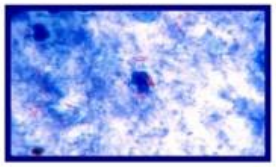


2-Carbol fuchsine and heating 5 minutes than wash with water.

3-Decolorize with acid-alcohol 95% for 15-20 second and wash with water.



4-Methylene blue or malachite green (counter stain) for 30 second and wash with water.



5-Examine with oil immersion lens.

3-Structural stains:

a- Endospore stain .

species of *Bacillus* and *Clostridium* produce a structure referred to as the endospore.

1- Schaeffer and fultons stain.

Staining Method

1- Prepare smear of Bacillus or Clostridium, and fix them with heat

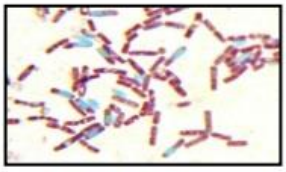
.

2-Cover the smear with 5% malachite green and continue heating for 5 minute.

3-Washing with water.

4- Safranin for 30 second , wash with water.

5- Examine under oil immersion lens.



2-Modified Ziehl-Neelsen stain.

Staining Method

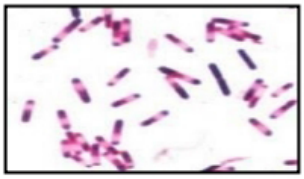
1- Prepare smear of Bacillus or Clostridium, and fix them with heat.

2-Cover the smear with carbol fuchsin and continue heating for 5 minute.

3-Washing with water.

4- Methylene blue for 30 second , wash with water.

5- Examine under oil immersion lens.



b- Capsular stain.

Some bacterial cell are surrounding by an extracellular slime layer called a capsule.

There are three main stain of capsule:-

1-Hiss stain. 2-Anthony stain. 3- Negative stain.

1-Hiss stain:-

Staining Method

1-Prepare smear and fixed.

2-Cover the smear with carbol fuchsin and continue heating for 5 minute.

3-Washing with 20% copper sulfate solution.

4-Examine under objective lens 40x.

2-Anthony stain.

Staining Method

1-Prepare smear and fixed.

2-Cover the smear with crystal violet and continue heating for 5 minute.

3-Washing with 20% copper sulfate solution.

4-Examine under objective lens 40x.

3- Negative stain.

There are two method of Negative capsule stain :-

1-Dry Negative stain.

Staining Method

1- two loopful of the organisms are mixed in a small drop of Indian ink.
2- Spread the suspension of bacteria over slide as a blood smear.
3-Dried with air and examine with oil immersion lens.

1-Wet Negative stain.

Staining Method

1- two loopful of the organisms are mixed in a small drop of Indian ink.
the suspension of bacteria with cover slide without dried --
2examine with oil immersion lens.

c- Flagellar stain:-

Solution of dye:

1- 1% Nacl.
2- 3% tannic acid.
3- 1.25% basic fuchsine dissolve with 95% ethanol.

Staining Method

Putting two loopful of the organisms on clean slide without mixed and dried
1- with air .

2-Cover the smear with Flagellar stain for 30 second, wash with water.

3- Methylene blue for 30 second , wash with water.