MEDICAL MICROBIOLOGY

Lab 2

Bacterial staining

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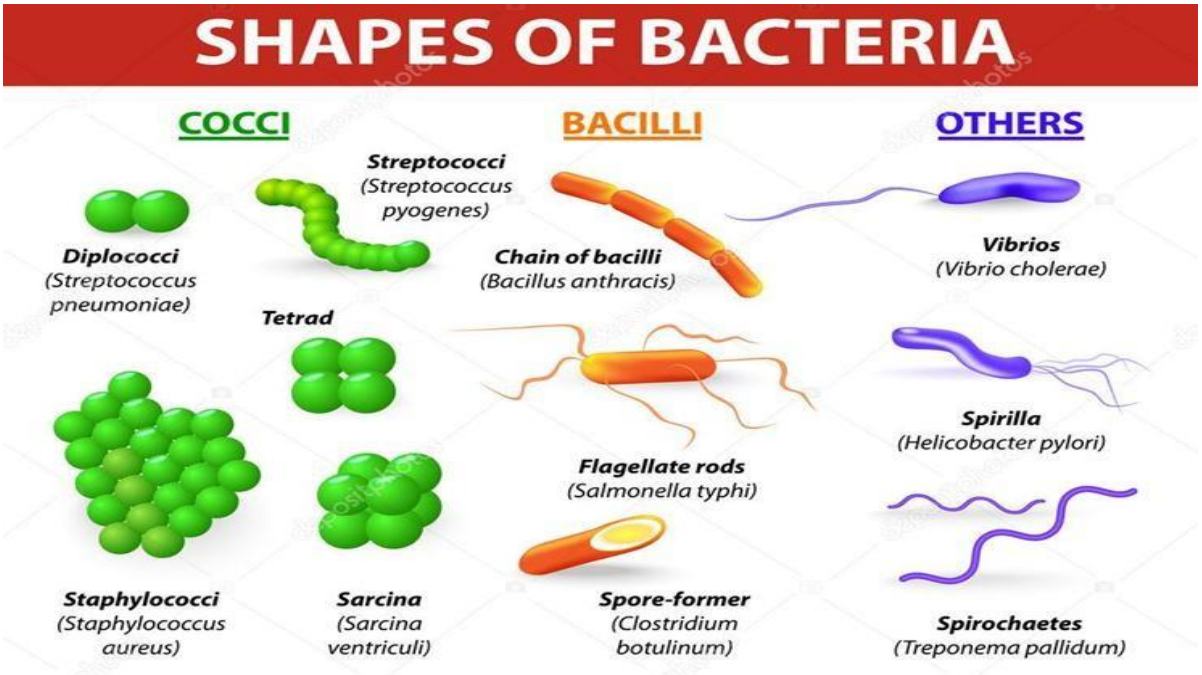
Bacterial Staining

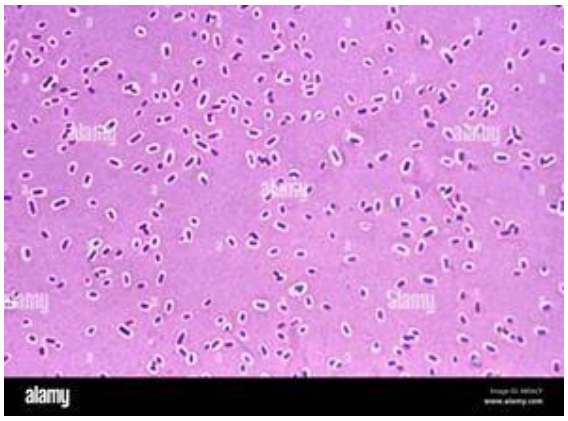
Staining : is the process of adding a dye to a bacterial culture . The staining allows to differentiate various bacterial morphology and arrangements , direct diagnosis of disease and observe certain structures in bacteria such as capsules , endospores and flagella .

Dyes may be divided into two general classes based on the nature of their charged group :

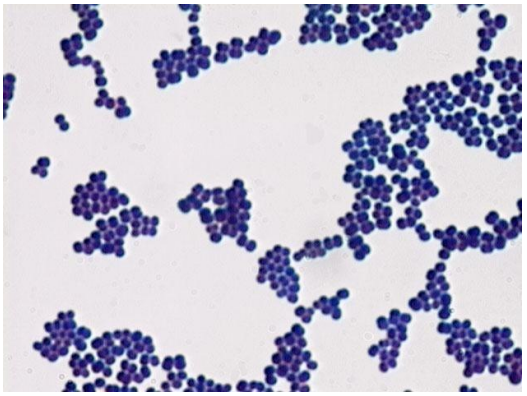
1- **Basic dyes (+)/ direct staining (positive staining)** : methylene blue , crystal violet ,basic fuchsin , safranin and malachite green - have positively charged groups . Basic dyes bind to negatively charged molecules of bacteria (opposite charges) and the organism directly stained .

2- **Acid dyes (-) / indirect staining (negative staining)** : because of their negatively charged is repelled by the negatively charged of the bacteria ex. India ink or nigrosin , stains the background , not the bacteria.





Negative staining



Positive staining

Types of staining techniques:

1-Simple staining : A staining method uses only a single staining step involves application of one stain to show shape and arrangement of cells. Simple staining includes two types:

A-Direct / positive staining : stain object

B-Indirect / negative staining : stain background

2-Differential staining (uses of two contrasting stains) is include Gram stain and Acid fast stain

3- Special stain (capsule stain ,spore stain and flagella stain)

.

Staining technique include smear , air dry , heat fix ,stains .

Smear preparation

A bacterial smear is a dried preparation of bacteria cells on a glass slide .

Fixation is the process by which the internal and external structures of cells are preserved and fixed in position , fixing is necessary to ensure that cells adhere to the slid and make organisims more readily accept stains .

prepare a Smear

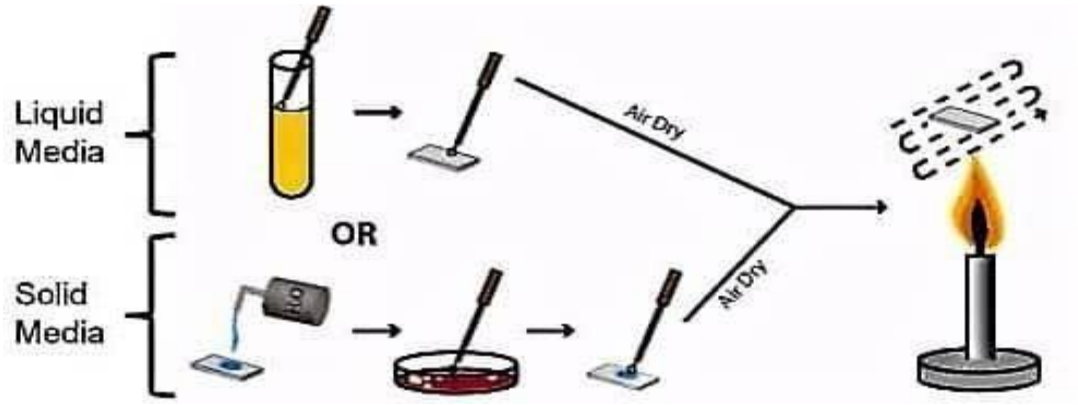
1-Transfer one or two loopfuls of water or saline on to the center of the slide.

2-Flame loop and allow to cool.

3- Using aseptic technique, transfer a very small part of a single colony from a plate or slant of agar medium into the water or saline . (If we use liquid culture the use of water to prepare the smear will be unnecessary) .

4-Make a suspension on the slide and gently spread it evenly over an oval area.

5-Dry the suspension by warming gently over a Bunsen burner flame and then fix it by quickly passing it through the flame a few times .



Simple staining

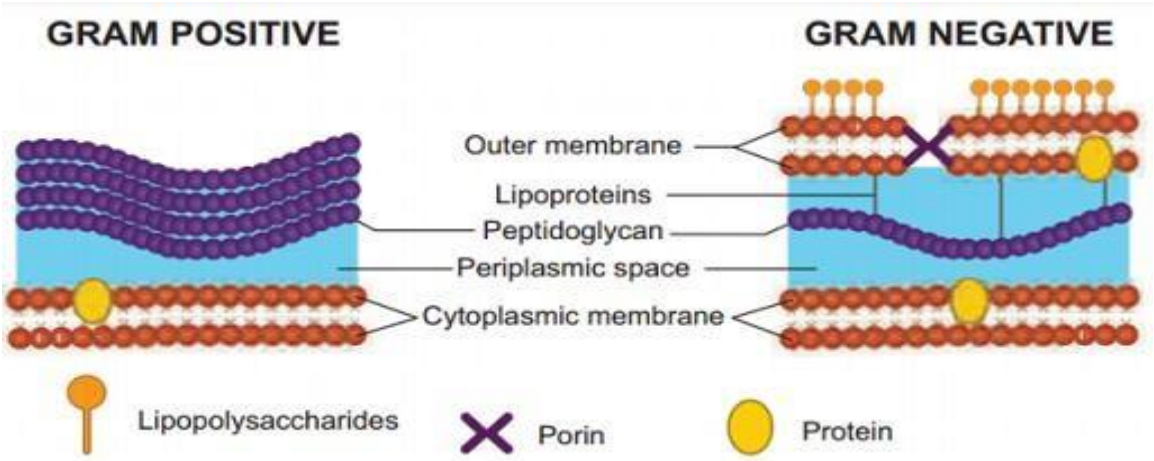
1-prepare a smear.
2-Cover smear with methylene blue for 1
minute . 3-Rinse the slide with distilled water .
4-Cover the slide with iodine for 1
minute . 5-Rince the slide with distilled
water .
6-Blot dry the smear and view slide under 100x oil
immersion .

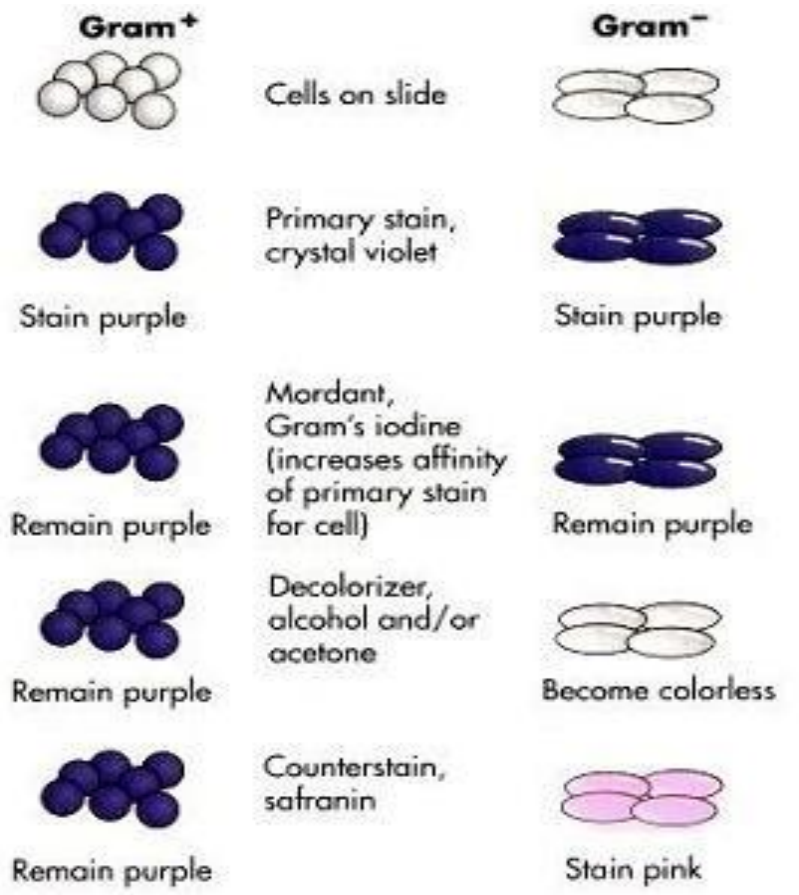
Gram staining

The Gram staining is the most important staining procedure used in bacteriology .It is a differential staining method . It differentiate bacteria into gram (+) and gram (-) according to the chemical and physical properties of their cell walls . Gram positive bacteria have thick (thick layer peptidoglycan) , dense , non – porous walls ,while Gram negative bacteria have thin walls(thin layer peptidoglycan) surrounded by lipid – rich memberanes .

Gram stain procedure

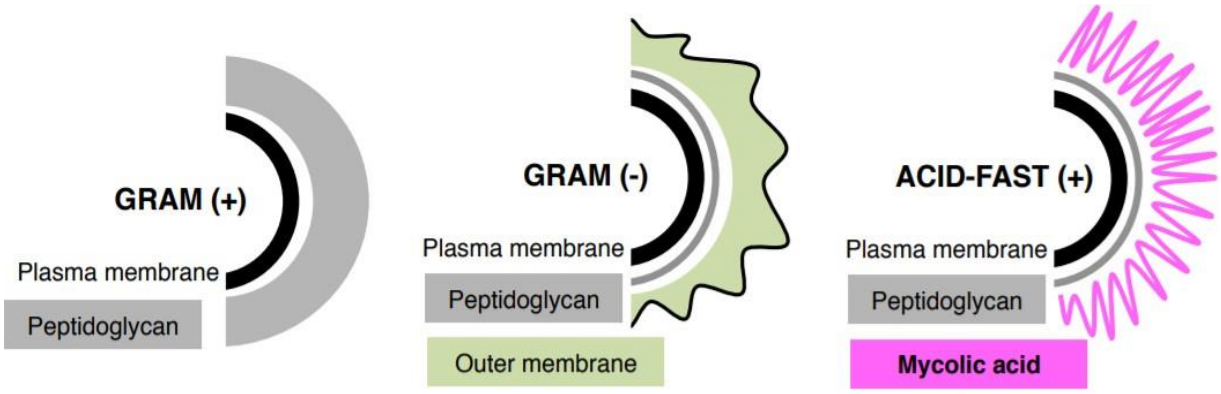
1-prepare a smear .
2-cover the smear with Crystal Violet stain (the primary stain) for 1 minute .
3-wash the slide with distilled water .
4-Cover the smear with Iodine (the mordant) for 1 minute .
5-Wash the slide with distilled water .
6-Decolorize with 95 % ethanol or ethanol / acetone for 2- 3 sec until the run –off is clear .
7-Cover the smear with safranin stain (counterstain) for 1 minute .
8- wash the slide with distilled water .
9- Blot dry the smear and view slide under 100x oil immersion.

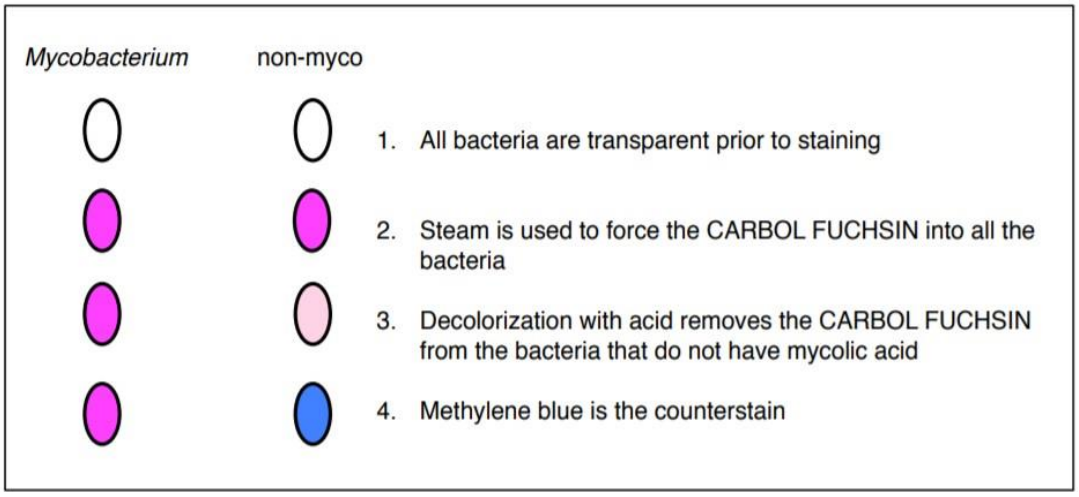




Acid fast stain (Ziehl-Neelsen method)

Is another important differential staining procedure used to detect the genus Mycobacterium (tuberculosis and leprae) , depends upon their lipid –rich cell walls ; in particular , mycolic acid – agroup of branched chain hydroxyl lipid – appears responsible for acid –fastness.

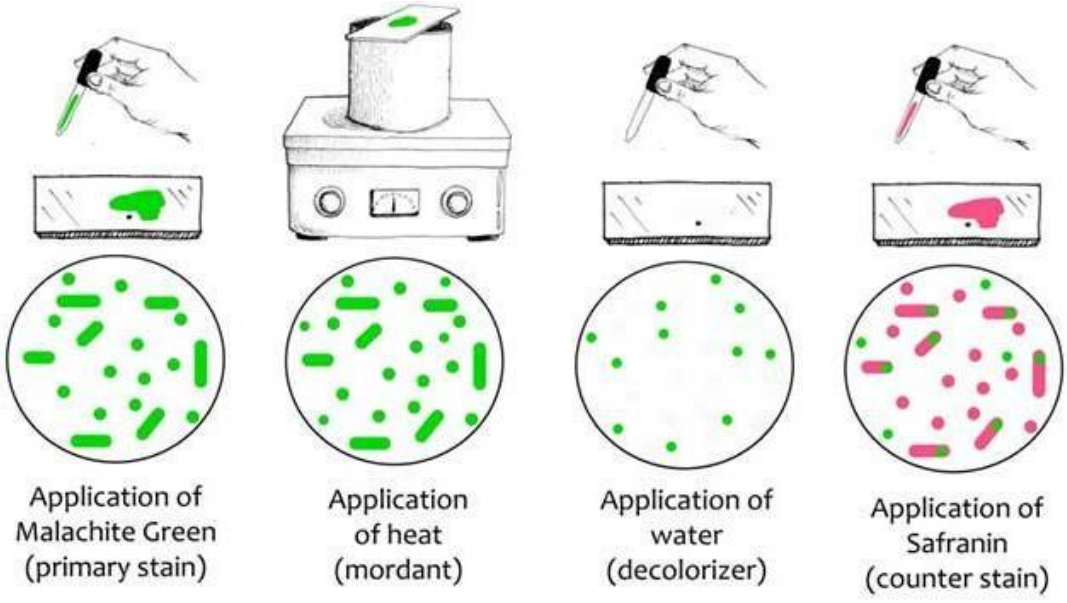


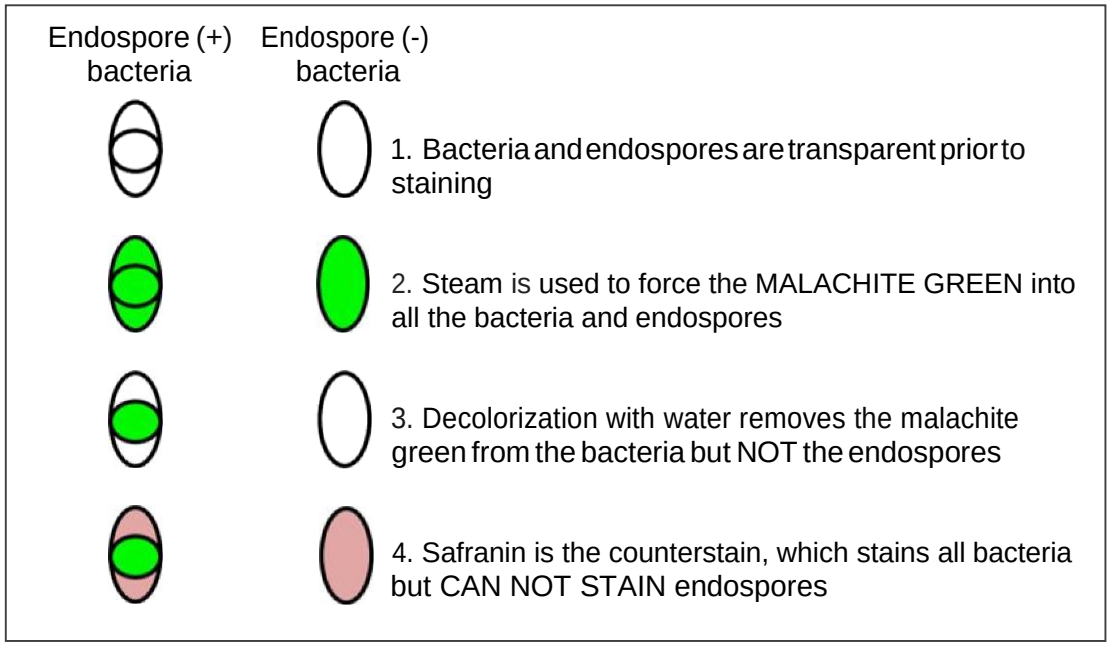


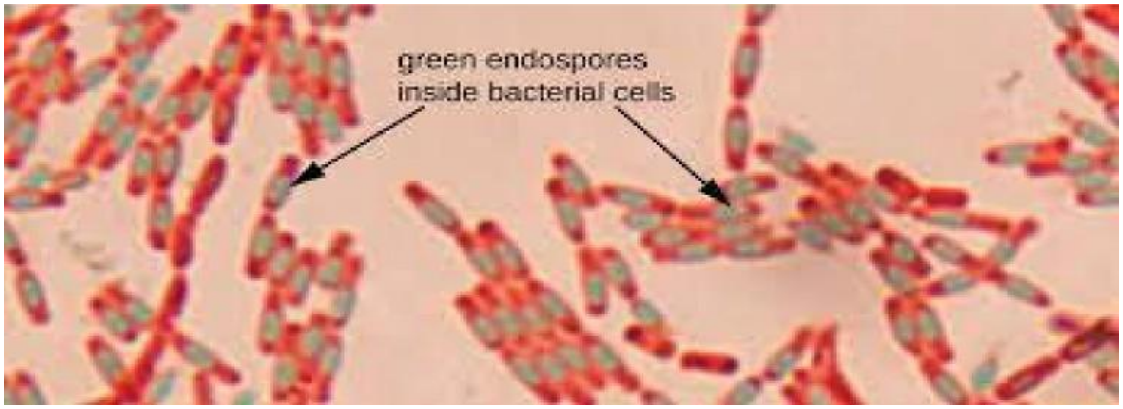
Endospore stain

Bacteria in the genera *Bacillus* and *Closrtidium* form a resistant structure capable of surviving for long periods in an unfavorable environment , this structure is called an endospore since it developes within the cell and they are spherical to oval in shape . Endospores are not stained well by most dyes, but once stained, they strongly resist decolorization. Endospore first stained by heating bacteria with malachite green which is a very stronge stain that can penetrate endospores.

Procedure of endospore stain




Endospore (+) bacteria
Endospore (-) bacteria
1. Bacteria and endospores are transparent prior to staining
2. Steam is used to force the MALACHITE GREEN into all the bacteria and endospores
3. Decolorization with water removes the malachite green from the bacteria but NOT the endospores
4. Safranin is the counterstain, which stains all bacteria but CAN NOT STAIN endospores


green endospores
inside bacterial cells

Capsule stain (Negativ stain)

Is a technique that reveals the presence of the diffuse capsule surrounding many bacteria . Bacteria are mixed with India ink or nigrosin dye and spread out in a thin film on a slide . After air

–drying , bacteria appear as lighter bodies in the midst of a blue

–black background because ink and dye particles cannot penetrate either the bacterial cell or its capsule . In this staining we use acidic dye (India ink or nigrosin) to stain the background of the slide and basic dye (methylene blue or crystal violet) to stain the cell .