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قسم :العلوم المختبرية السريرية

اسم المادة باللغة العربية:احياء مجهرية طبية I

اسم المدة باللغة الإنكليزية:**Medical Microbiology I**

المرحلة:الثانية

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عنوان المحاضرة باللغة العربية:التصبيغ

عنوان المحاضرة باللغة الإنكليزية: **Examination of stained microorganisms,**

Smear preparation simple staining, Gram staining and Acid-fast staining

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Examination of stained microorganisms, Smear preparation, simple staining, Gram staining and Acid-fast staining

Why we need to stain bacteria?

Bacteria are transparent and colorless so they would be invisible to naked eye if observed under microscope thus bacteria should be stained with certain dyes in order to visualize bacterial cell or their internal structures using the light microscope.

Terms related to staining

A stain is a substance that adheres to a cell, giving the cell color. The presence of color gives the cells significant contrast so they are much more visible. A stain is a dye consisting of a colored organic compound in the form of salt composed of positive and negative ion, one of these ions is responsible for colour (a chromophore)

Staining

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biology and medicine.

Based on the charges:

A stain is classified in to:-

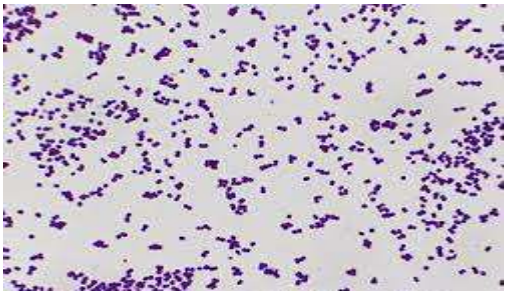
- Basic stain/dyes – stain with +ve charge, example include crystal violate, methylene blue and safranin
- Acidic stain/dyes – stain with –ve charge, example nigrosin and India ink.
- Neutral stain/dyes – stain with both charges.

Based on function of stain:

1. **Simple staining –** A staining method that uses only a single dye that which does not differentiate between different types of organisms- There is only a single staining step and everything is stained with the same color Simple stains are used to stain whole cells or to stain specific cellular components. Types of simple staining:-

   - . Direct / Positive staining : stain object

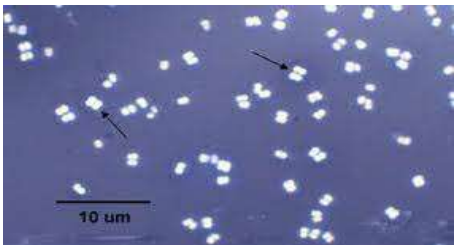
   A simple staining technique that stains the bacterial cells in a single color. Many of the bacterial stains are basic chemicals; these basic dyes react with negatively charged bacterial surface (opposite charges attract) and the organism becomes directly stained. Examples are methylene blue, crystal violet, and basic fuchsin.



Staphylococcus aureus stained with crvstal violet

- Indirect / Negative staining: stain background

  In this staining process, instead of cells background is stained Here, an acidic dye like nigrosin or Indian ink is used. Acidic stain carries a negative charge and repelled by the bacteria, which also carry a negative charge on their surface. Hence, an acidic dye do not stain bacteria, instead, it forms a deposit around the organism, leaving the organism itself colorless or transparent upon examination.



Negative staining

2. **Differential staining-** is a procedure where more than one dye is used to differentiate between different types of microorganisms on a slide. This type of staining helps to differentiate between cell types and cell structures.- Eg. Gram's staining, Acid-fast staining.

3. **Special staining –** more than one dye used -Special structures are seen. Eg. Capsule staining, Spore staining

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Principle of staining:

- **Each staining methods have own principles but the following steps may be common:**

❖ **Basic stain(+ve charge)**
- **To stain -ve charged molecules of bacteria**
- **Mostly used because cell surface is –ve charge.**

❖ **Acidic Stain(-ve charge)**

- **To stain +ve charged molecules of bacteria.**
- **Used to stain the bacterial capsules**

What meanings of bacterial smear and how can prepare???

Bacteria are cultured using either agar or broth media. After cultivation, bacteria are transferred to a glass slide for staining. The preparation of bacterial smear before staining called fixation

Requirements:

1- Bacterial growth (liquid or solid media)
2- Inoculating loop.
3- Clean glass slide.
4- Bunsen burner.

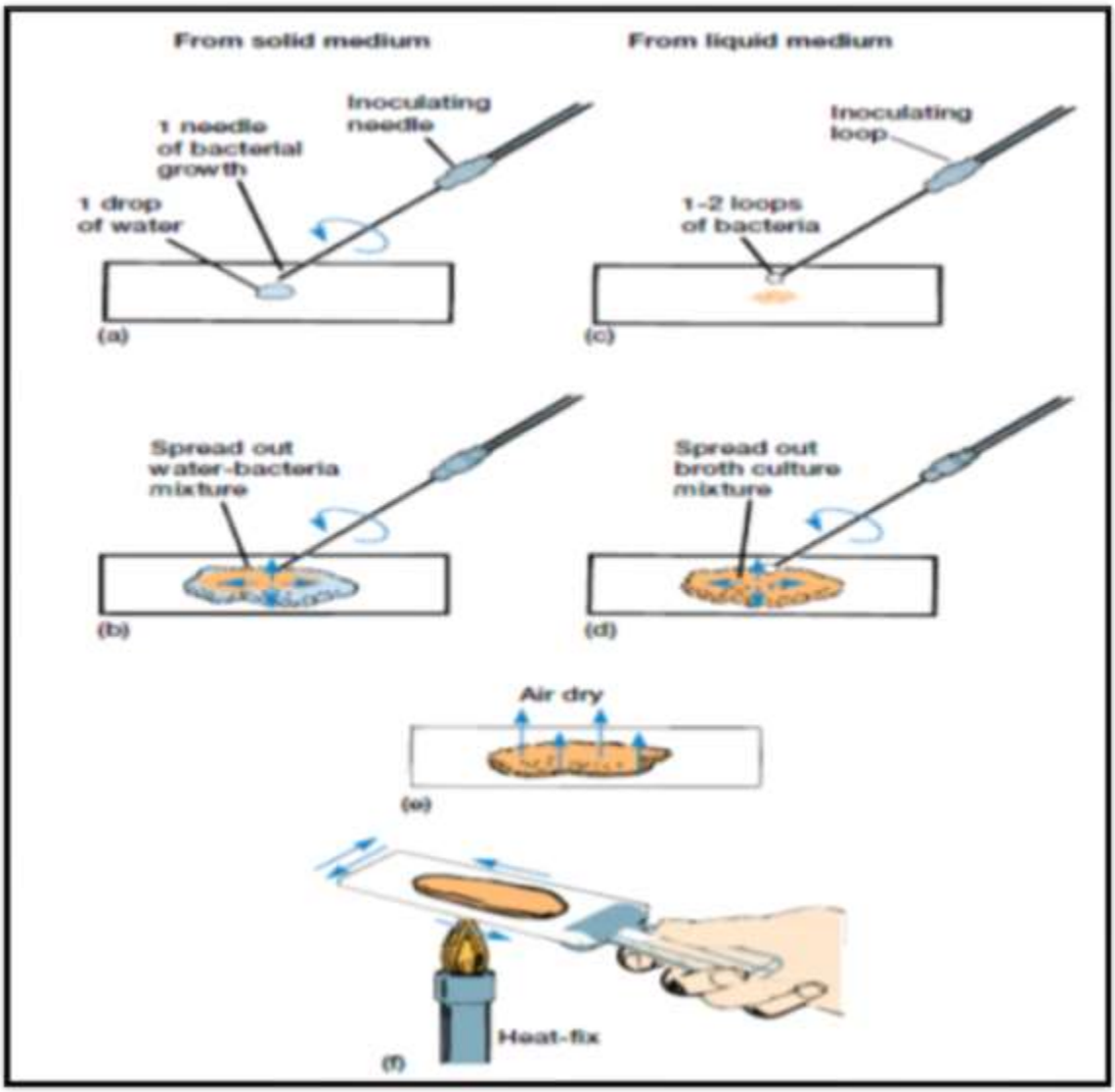
How do you prepare a smear?

A- From liquid media:

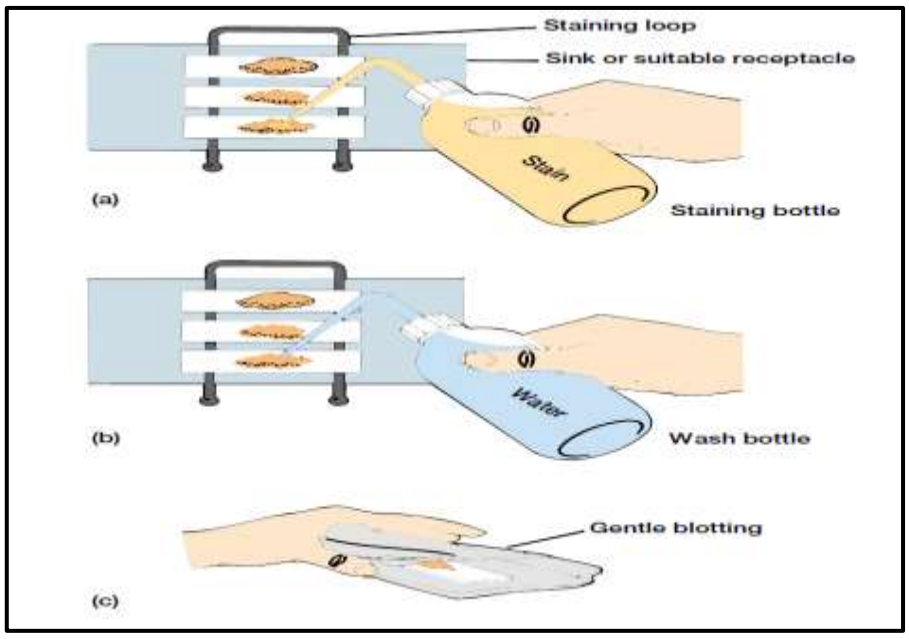
1- Sterilize the loop by Bunsen flame then let it cool.
2- Shake the specimen container (broth culture) then withdraw one or more if needed loopful from the specimen and spread it on the center of a clean slide to form a thin film of 1- 2 cm in diameter, then sterilize the loop.
3- Allow the smear to dry by air.
4- The smear fixed by passing it (3- 4) times through the Bunsen flame then allow the slide to cool before staining.

B- From solid media (slant or plate):

1- Sterile the loop on Bunsen flame and let it cool.
2- Place a drop of clean water on the center of a clean slide.
3- Resterilize the loop, transfer a small portion of the growth, mix it with water thoroughly and spread the mixture evenly on the slide to form a thin film of 1- 2 cm in diameter.
4- Dry and fix (as mentioned above)



Fixation procedure



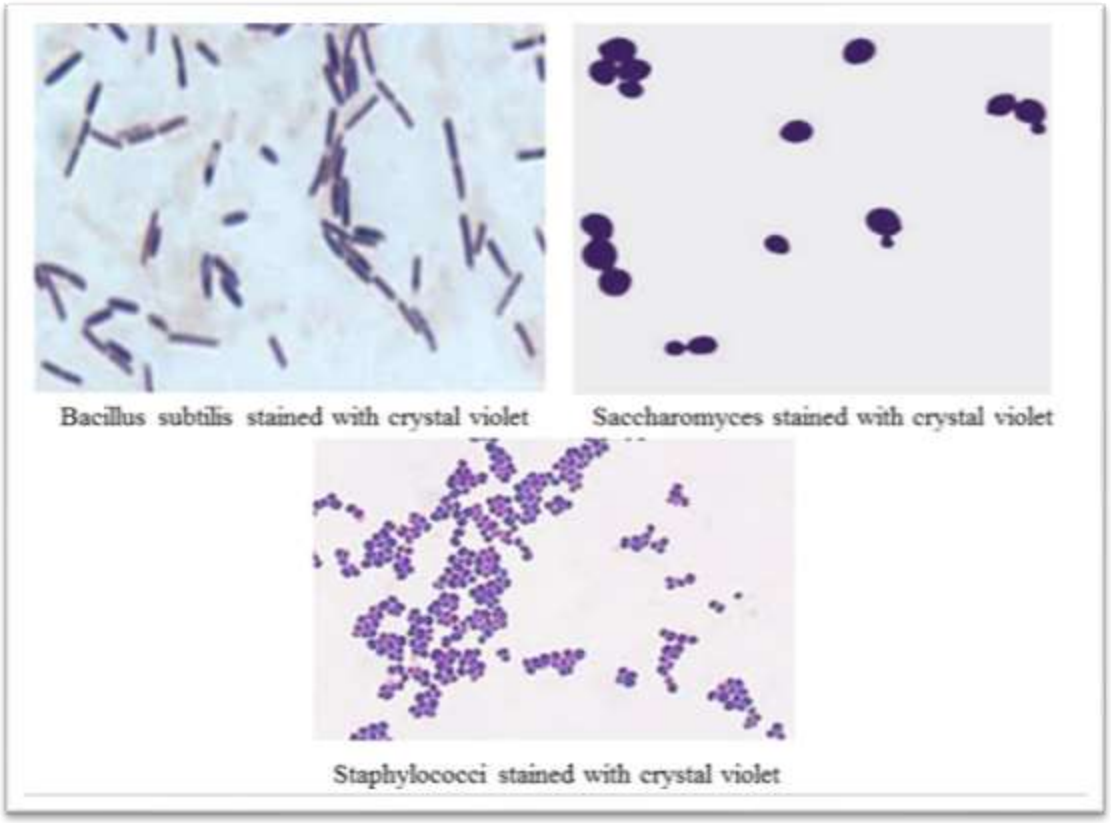
Simple staining

Simple stains use a single basic stain to color bacteria cells so that their size, shape, and arrangement can be observed.

The procedure:

Places the heat fixed smear on a staining rack, covers the smear with a small amount of the desired stain (crystal violet, carbol fuchsin and methylene blue) for the proper amount of time, washes the stain off with water for a few seconds then let it dry and examine under the microscope.

Simple staining procedure



Differential stains

Differential stains, such as Gram stain and acid-fast stain differentiate bacteria based on the chemical composition of their cell wall. Differential stains use two stains instead of one. The first stain is called the primary stain, while the second one is called the counterstain. A decolorization step occurs between application of the primary stain and the counterstain.

Depending on the composition of the cell wall, bacteria will either retain the primary stain during decolorization or lose the primary stain and take up the counterstain.

Differential stains include:

1- Gram staining

Most bacteria possess a cell wall that contains either a thick peptidoglycan layer or a thin peptidoglycan layer with an additional lipopolysaccharide layer. This chemical difference is distinguished with the Gram stain.

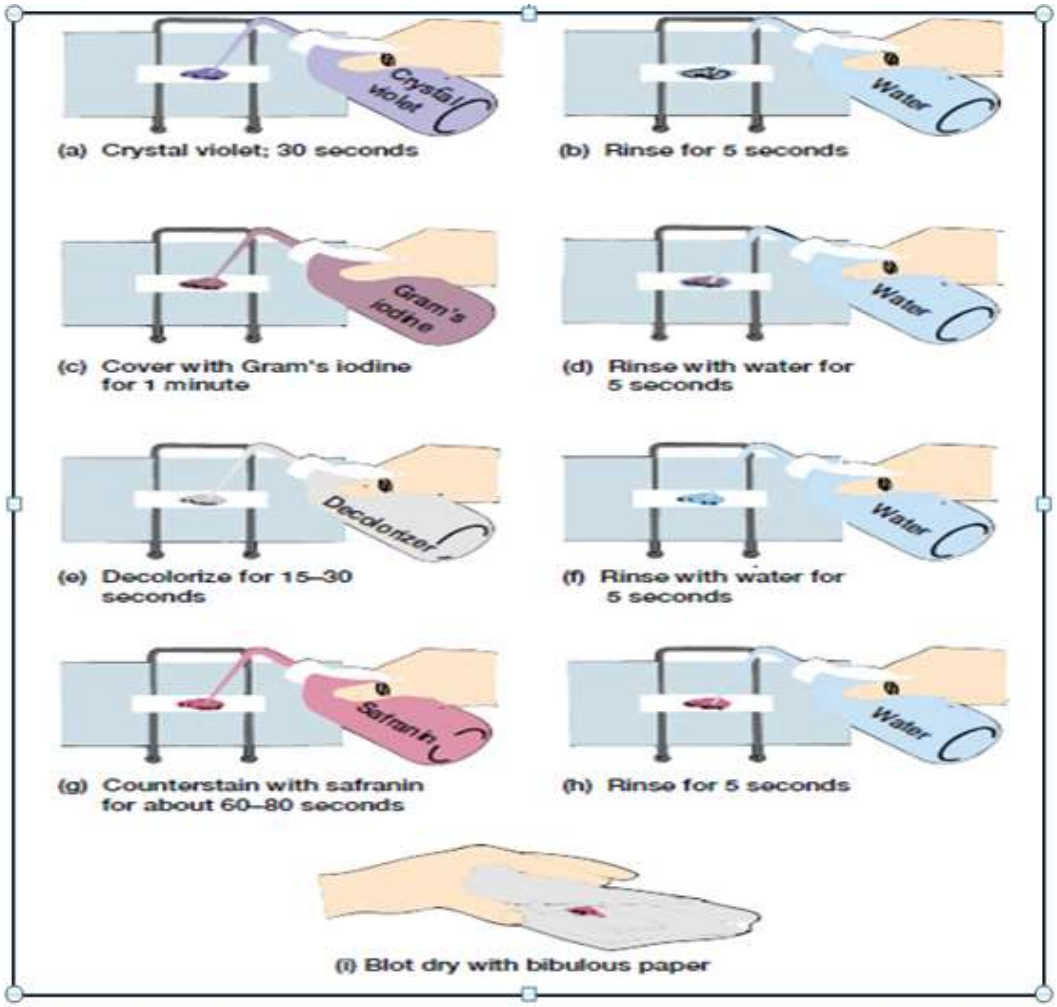
Requirements – staining reagents:

1. Crystal violet – Primary stain :-to stain cells
2. Gram's iodine- mordant/fixative
3. Acetone (95%)- decolorizer
4. Safranine/dilute carbol fuchsin – counterstain:- to mark all cells

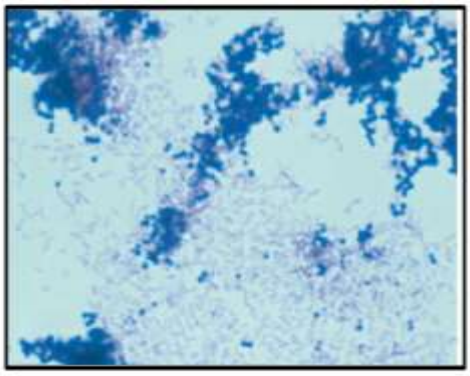
Procedure for Traditional Gram-Stain Technique:-

1. Prepare heat-fixed smear.
2. Place the slides on the staining rack.
3. Flood the smears with crystal violet and let stand for 1 minute.
4. Rinse with water for 5 seconds.
5. Cover with Gram's iodine mordant and let stand for 1 minute.
6. Rinse with water for 5 seconds.
7. Decolorize with 95% ethanol for 15 to 30 seconds
8. Rinse with water for 5 seconds.
9. add counterstain (safranin) for about 1minute.
10. Rinse with water for 5 seconds.
11. Let the smear dry and examine under oil immersion.

Gram-positive organisms stain blue to purple; while, gram-negative organisms stain pink to red.



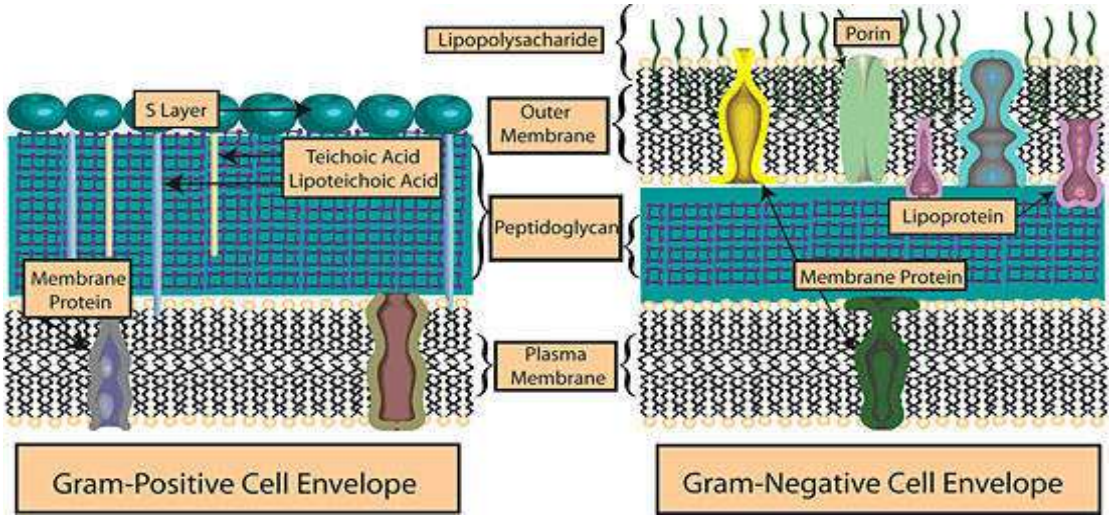
Gram staining procedure



Light micrograph (X900) of a gram stained mixture of gram positive Staphylococcus aureus (purple cocci) and gram negative Escherichia coli (pink rods)

What is the different between Gram positive and Gram negative bacteria?

Gram positive species have a thick peptidoglycan layer and large amount of teichoic acid, lipid and lipoprotein content, which is low and are therefore unaffected by alcohol decolorization and retain the initial stain (crystal violet) giving the organism violet appearance. On the other hand, Gram negative cell wall has a thin peptidoglycan layer with high lipid content. The outer membrane of Gram negative bacteria is damaged by alcohol decolorizer allowing crystal violet- iodine complex to take out and be replaced by the counter stain (safranin) giving the organism a pink/ red appearance.



1- Acid- fast stain

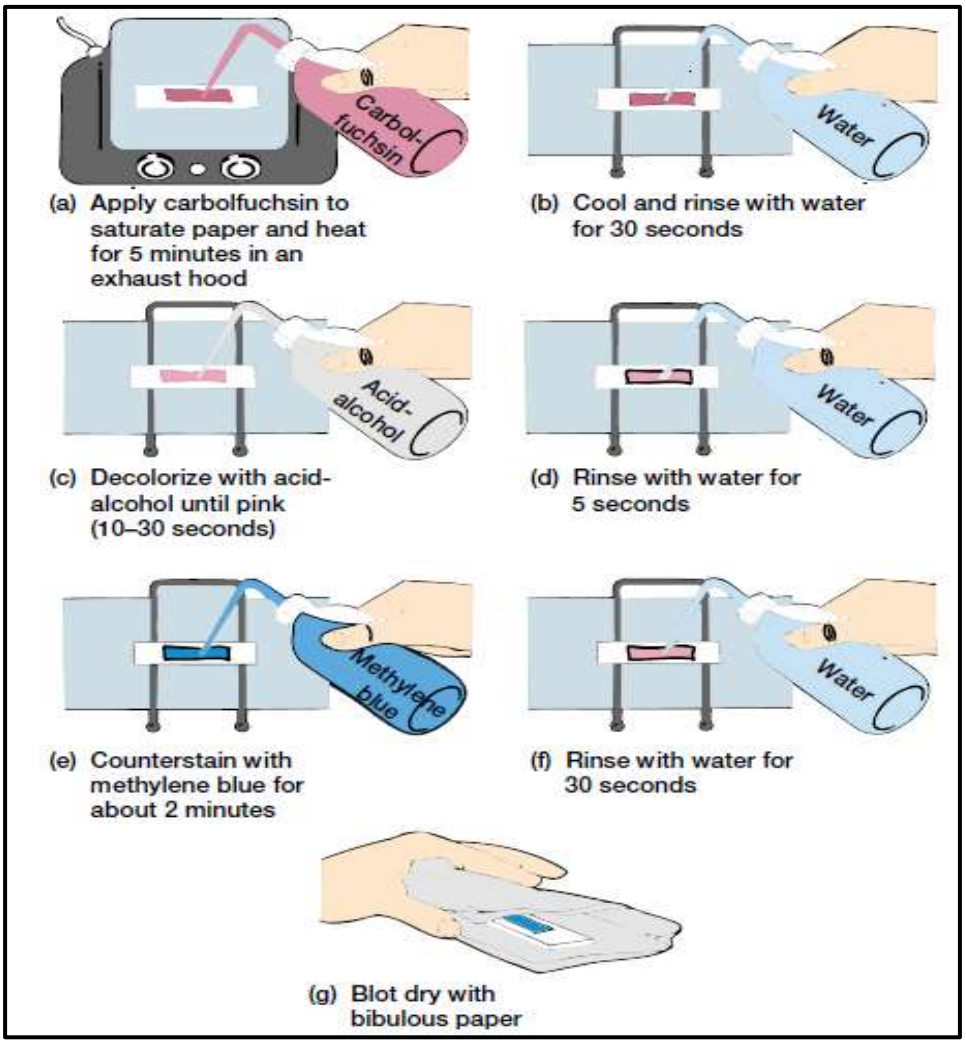
Acid fast stain is one of the most medically important stains. It used for acid- fast bacteria, such as *Mycobacterium tuberculosis* and *mycobacterium leprae*, which contain long chain fatty acids (mycolic acid) in their cell walls. The method that used for acid fast stain, the Ziehl Neelsen method**.**

The acid fast stain distinguishes different type of bacteria based on the wax content of their cell wall. Bacteria with high wax content retain the primary stain carbolfuchsin when decolorized with acid- alcohol. These bacteria are called acid- fast. On the other hand, bacteria with low

wax content in their cell wall lose carbolfuchsin when decolorized and take up the counterstain methylene blue. These bacteria are called non-acid fast.

Procedure

1- Prepare a smear.
2- Allow the smear to air dry and then heat- fix.
3- Place the slide on a hot plate then added carbolfuchsin. Heat for 3 to 5 minutes but do not allow the slide to dry out and avoid excess flooding. Also, prevent boiling by adjusting the hot plate to proper temperature.
4- Remove the slide, let it cool and rinse with water for 30 seconds.
5- Decolorize by adding acid- alcohol drop by drop until the slide remains only a slightly pink. This requires 10 to 30 seconds and must be done carefully.
6- Rinse with water for 5 seconds.
7- Add counterstain (alkaline methylene blue) for about 2 minutes.
8- Rinse with water for 30 seconds.
9- Let the smear dry and examine the prepared slide under the microscope.



Acid fast staining procedure