جامعة الانبار

كلية: الصيدلة

قسم: العلوم المختبرية السريرية

اسم المادة باللغة العربية: الاحياء المجهرية

اسم المدة باللغة الإنكليزية: **microbiology**

المرحلة: الثانية

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عنوان المحاضرة باللغة العربية: تصبيغ البكتريا

عنوان المحاضرة باللغة الإنكليزية: **Bacterial Staining**

محتوى المحاضرة

Bacterial Staining

INTRODUCTION:

- Microbial Staining – giving color to microbes.
- Because microbes are colorless and highly transparent structures.
- Staining – process in which microbes are stained.

Why were we staining bacterial cells?

Staining bacterial cells for microscopic examination makes it possible to study their unique characteristics, including cell size, shape, arrangement and structure. You can use these characteristics for bacterial identification.

Stain and Staining

Stains/dyes - organic compounds consisting of a colored ion (a chromophore which carries either positive charges or negative charges or both) and a counter ion to balance the charge. Attachment of the dye complex to cellular components represents the staining reaction.

Classification of stains

- **Based on the charges:**

1**. Basic stain: +ve charge.**
  - To stain -ve charged molecules of bacteria
  - Mostly used because cell surface is –ve charge.
  - Eg: crystal violet, methylene blue and safranin.

2. **Acidic stain: –ve charge.**
  - To stain the background surrounding negatively charged bacterial cells, so you can see the cells in outline.
  - Eg: congo red, nigrosin and india ink.

3. **Neutral stain: both charges**

- **Based on function of stain:**

1. Simple staining – only one dye

Uses: To study morphology and arrangement of bacteria.

Eg. methylene blue and safranin.

2. Differential staining - more than one dye

Uses: Differentiation among bacteria is possible

Eg. Gram's staining and Acid-fast staining.

3. Special staining – more than one dye

Uses: Special structures are seen.

Eg. Capsule staining and Spore staining.

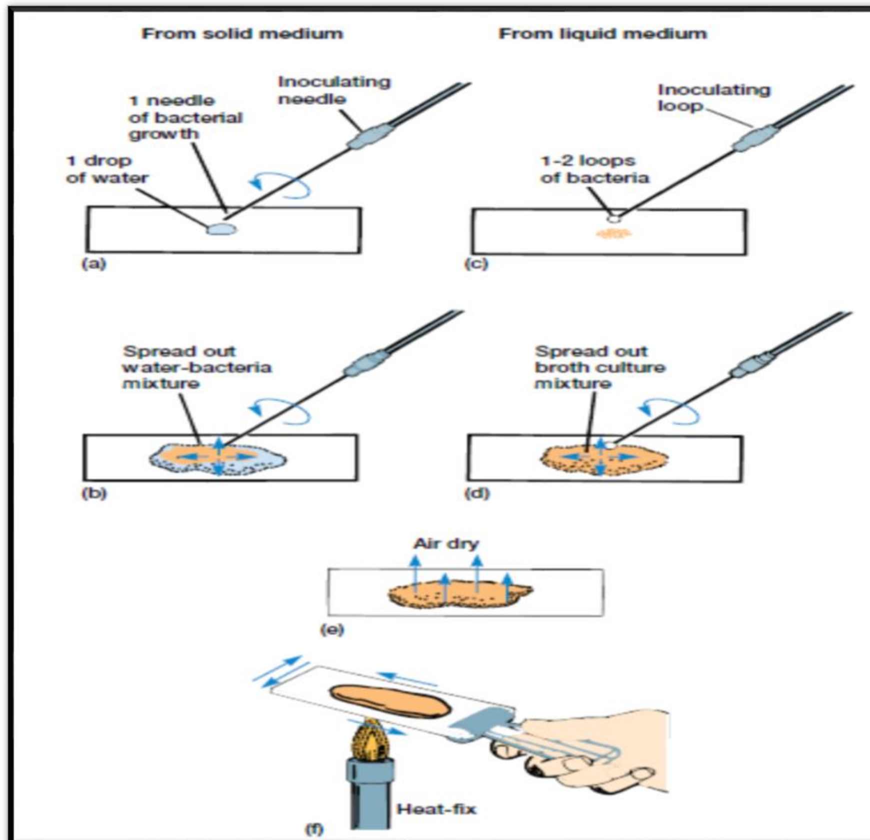
Basic requirements for staining:

- Clean grease-free slide .
- Bacteria to be stained .
- Inoculating loops- to transfer bacterial suspension to slide .
- Bunsen burner – to sterilise inoculating loops before and after smear preparation .
- Pencil marker – to mark the face of slide where bacterial smear is applied.

Basic initial step before staining:

Smear preparation:

- ✓ Putting of bacterial suspension (bacteria in liquid) to be stained on the central portion of slide in a circular fashion.
- ✓ Allow the smear to dry by air.
- ✓ The smear fixed by passing it (3- 4) times through the Bunsen flame then allow the slide to cool before staining.



Heat fixing will be:
*kills the organisms,
*makes them adhere to the slide,
*and permits them to accept the stain.

Simple Staining:

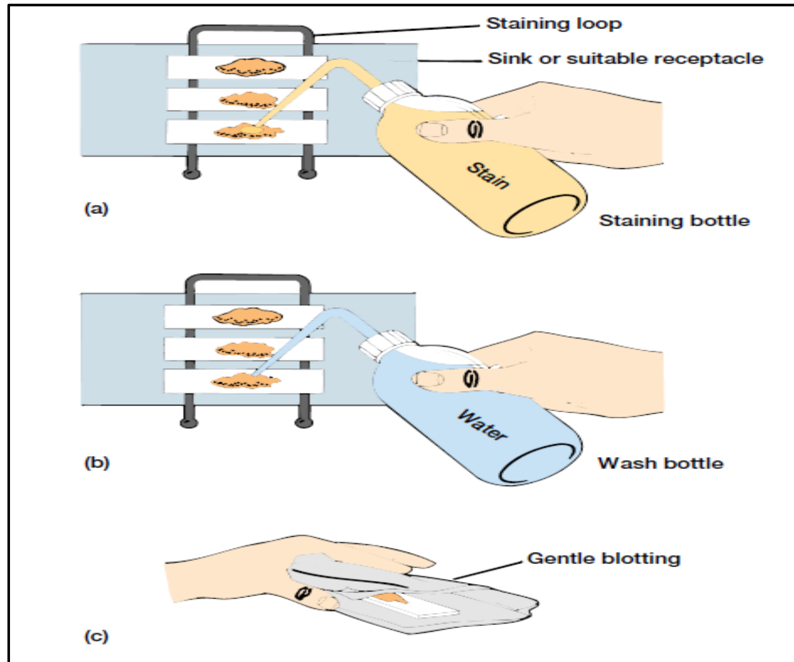
Simple = only one dye is used during the staining procedure.

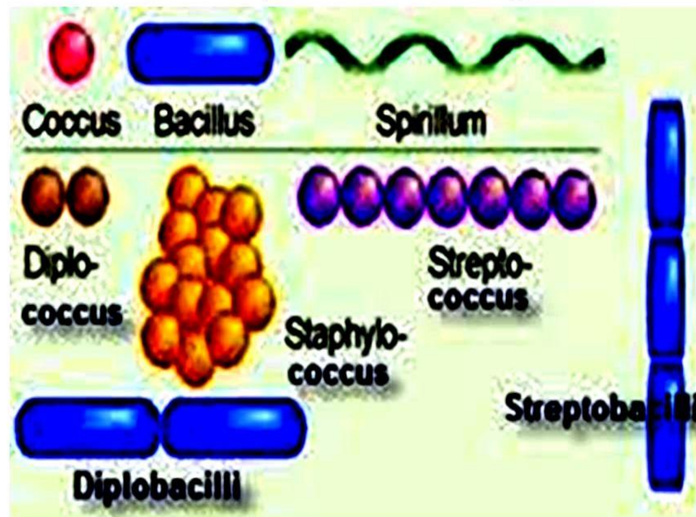
Procedure:

a. Places the heat fixed slide on a staining rack, then covers the smear with a small amount of the desired stain for the proper amount of time.
b. Washes the stain off with water for a few seconds.
c. Air dried and focused with 10x,45x & 100x ..

Results:

Morphology – spherical / rod /spiral.
Arrangement – clusters/chains.





Bacterial Shapes and Arrangements

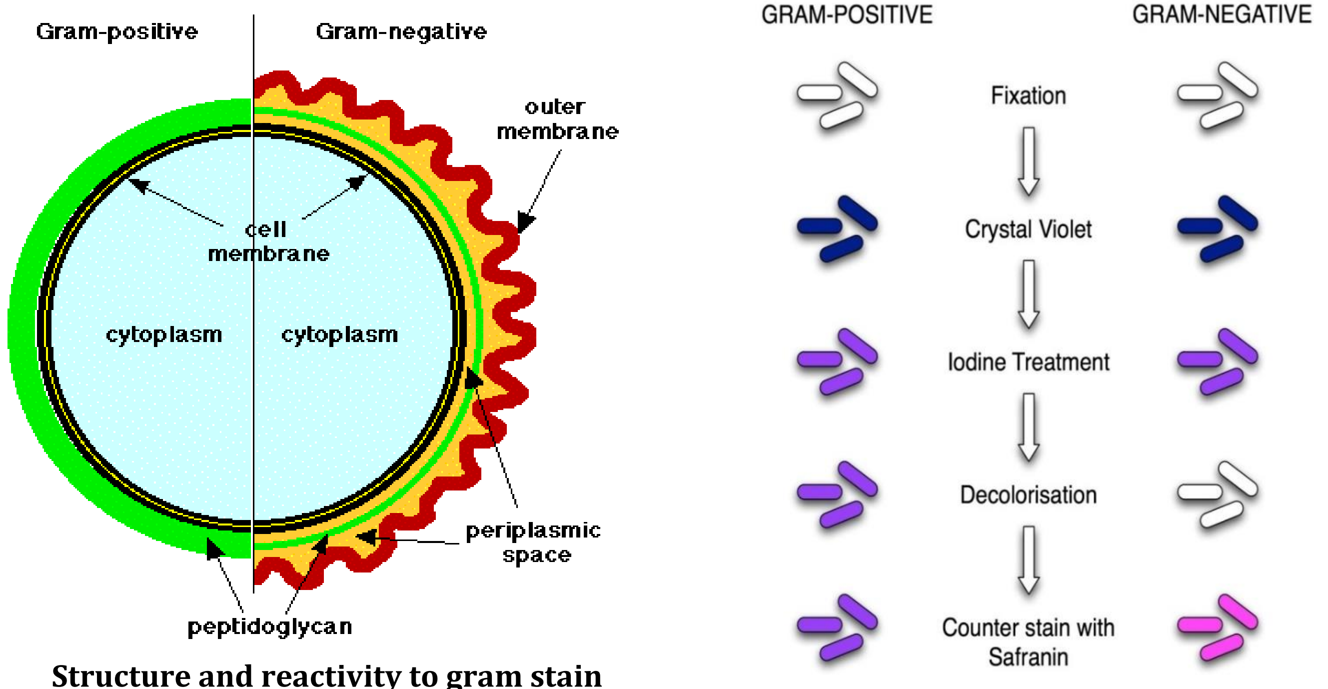
Differential stains

Differential stains, such as the 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, and the second is called the counter stain. 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. Based on this reaction, bacteria classified into Gram positive and Gram negative bacteria. The cell retains ether primary stain (G+) so it appears (blue or purple) or the counter stain (G-) it appears (pink to red) depend on wall composition.

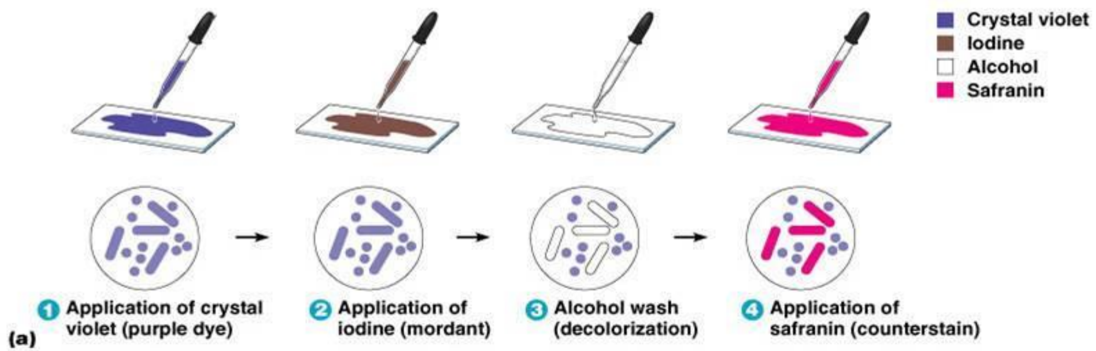


Structure and reactivity to gram stain

Requirements – Staining Reagents:

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

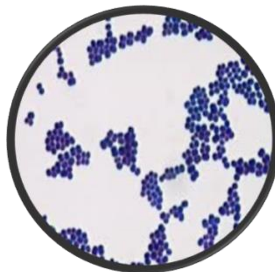
Procedure for Gram-Stain Technique



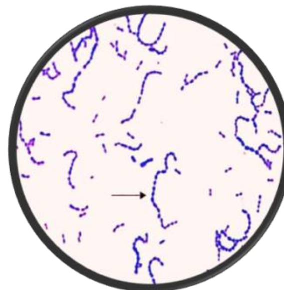
Time Frame

1) 1 minute
2) 1 minute
3) 15 seconds
4) 1 minute

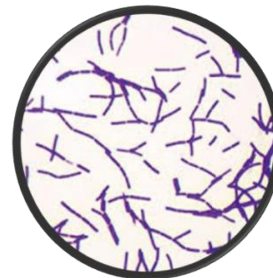
- Rinse with water between each step



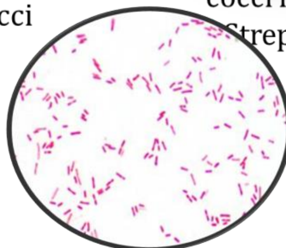
Gram positive cocci in clusters: Staphylococci



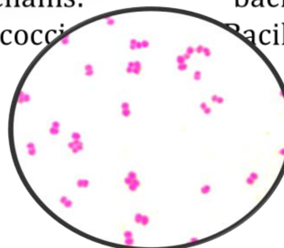
Gram positive cocci in chains: Streptococci



Gram positive bacilli in chains: Bacillus anthracis



Gram negative bacilli: Escherichia coli



Gram negative cocci: Neisseria species

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 and are therefore unaffected by alcohol decolourization and retain the initial stain (crystal violet) giving the organism violet appearance. On the other hand, Gram negative cell wall has a single peptidoglycan layer. The outer membrane is damaged by alcohol decolorizer allowing crystal violet- iodine complex to take out and be replaced by the counter stain (safranine) giving the organism a pink/ red appearance.

2- Acid- fast stain (Ziehl-Neelsen stain)

- To stain Mycobacterium species especially *M. tuberculosis.*
- High lipid content – makes decolorization very difficult.
- **Principle:**
- Acid fast (resist) – Property of Mycobacterium species - once these bacteria stained with primary dye – difficult to decolorize with acid.
- This property due to Mycolic acid in cell wall.

Staining Reagents:

1) Strong carbol fuchsin – primary stain
2) 20% sulphuric acid/3% Hcl – decoloriser – acid-fast property.
3) 95% alcohol- decoloriser- alcohol – fast property
4) Methylene blue/ Malachite green- counterstain.

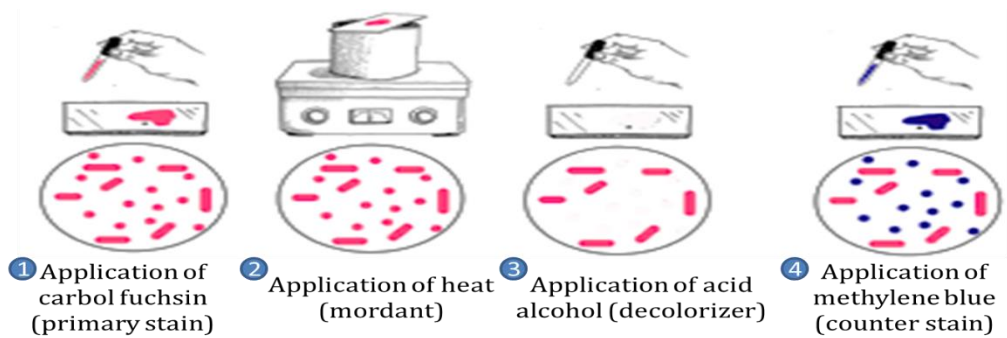
Note:

5% sulphuric acid – for M.leprae.

1% sulphuric acid – for Nocardia species.

Procedure

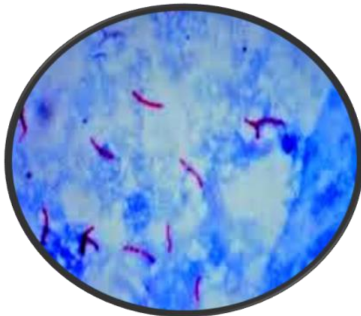
- Strong carbol fucshin-heat till steam rises – allow 5-10 min to act– wash.
- Decolorize with acid-alcohol mixture till get a faint pink color in the smear (take 3-5 min) – wash.
- Methylene blue/Malachite green – 2 min – wash.
- Allow to dry and focus under microscope.



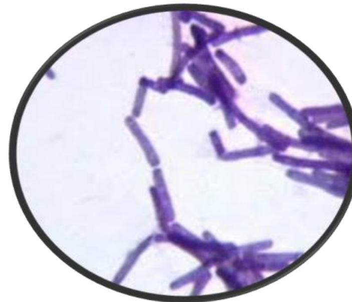
Time Frame

1+2) 5-10 minutes
3) 3-5 minutes
4) 2 minutes

- Rinse with water between each step



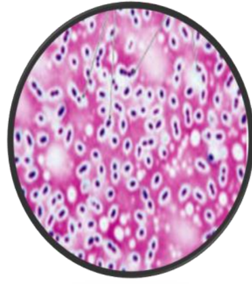
Pink bacilli – Acid fast bacteria/bacilli
M. tuberculosis



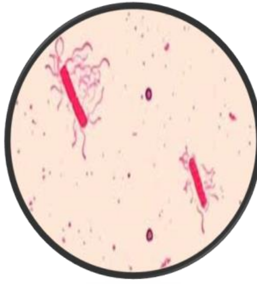
Blue colored bacteria – Non-acid fast

Special Stain:

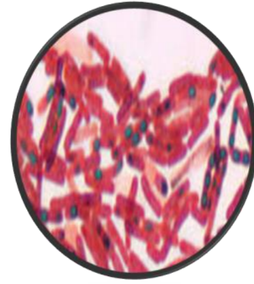
- Used to stain special structures of bacteria– capsule, spores, flagella, metachromatic granules.
- **Examples of Special Stain:**
  1) Capsule Stain - Nigrosin ink+ indian ink
  2) Spores - malachite green + safranin
  3) Flagella - RYU stain



Capsule Stain



Flagella stain



Spores stain