examination of stained microorganisms

Presented by:

Assist. Instructor:

Sulaiman A. Abdullah

College of pharmacy

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.
- We 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 a 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

Classification of stains

- **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:

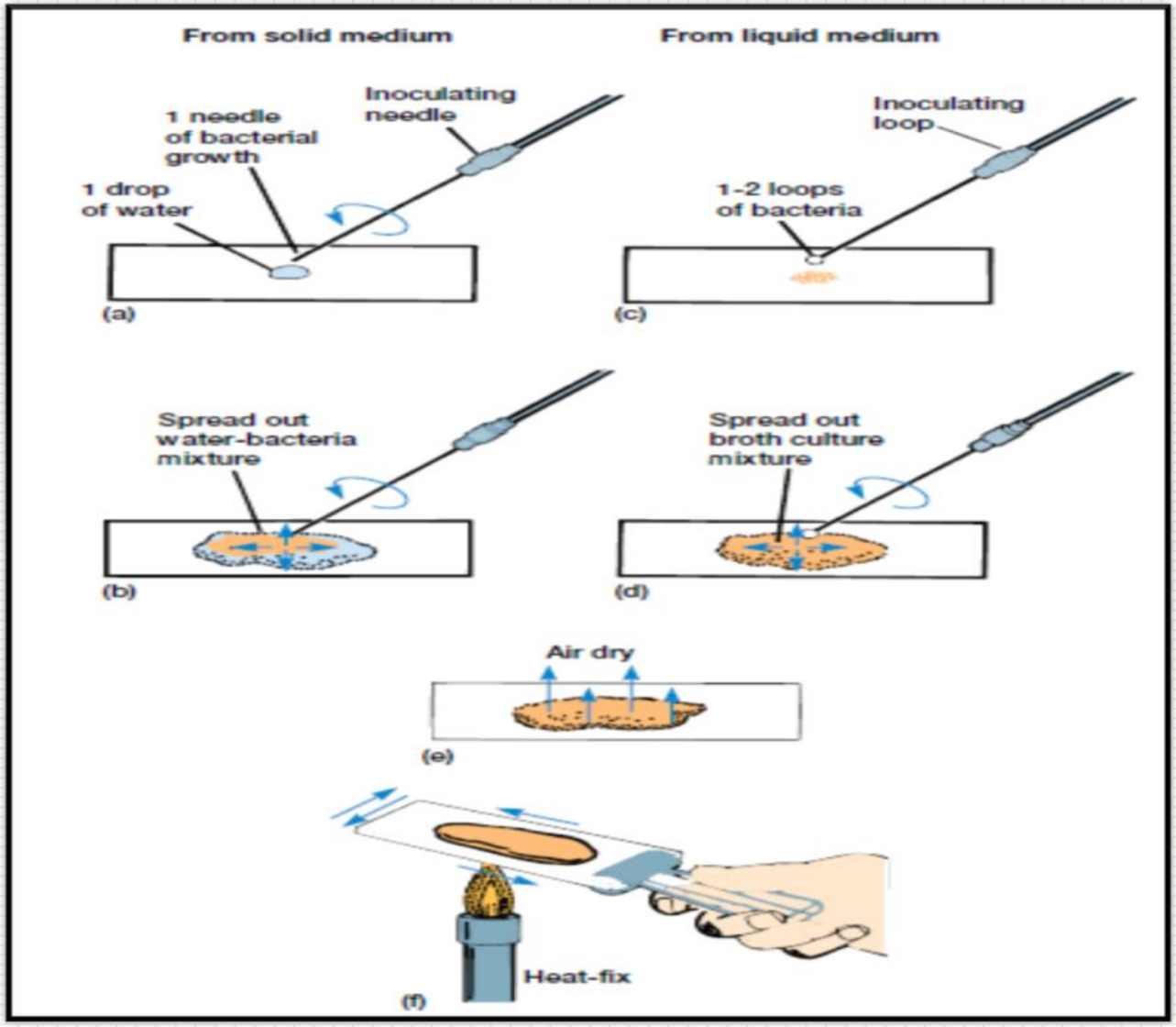
- **Smear preparation:**

✓ 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.

Smear preparation:



Simple Staining:

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.

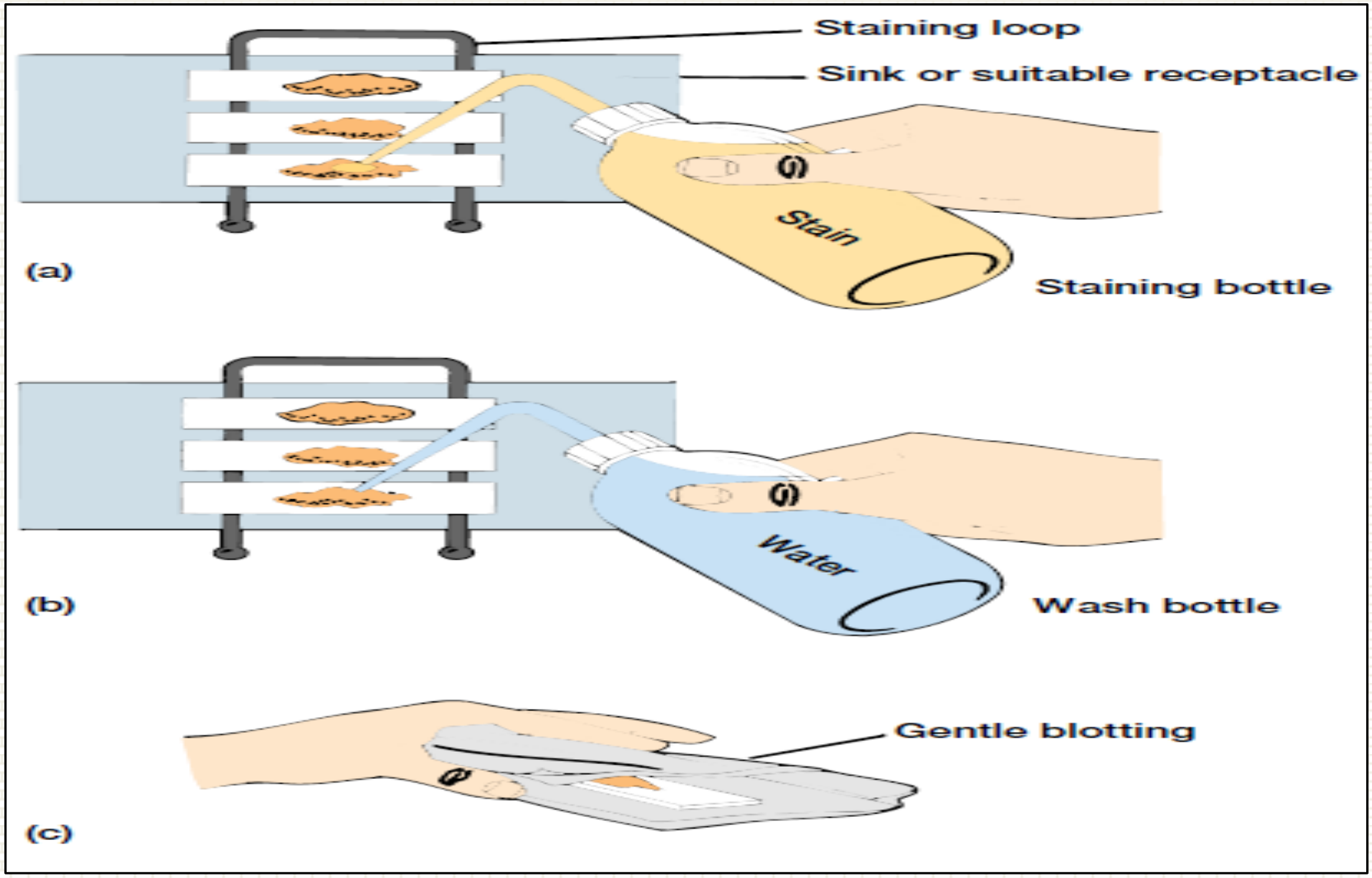
c. Air dried and focused with 10x,45x & 100x ..

Results:

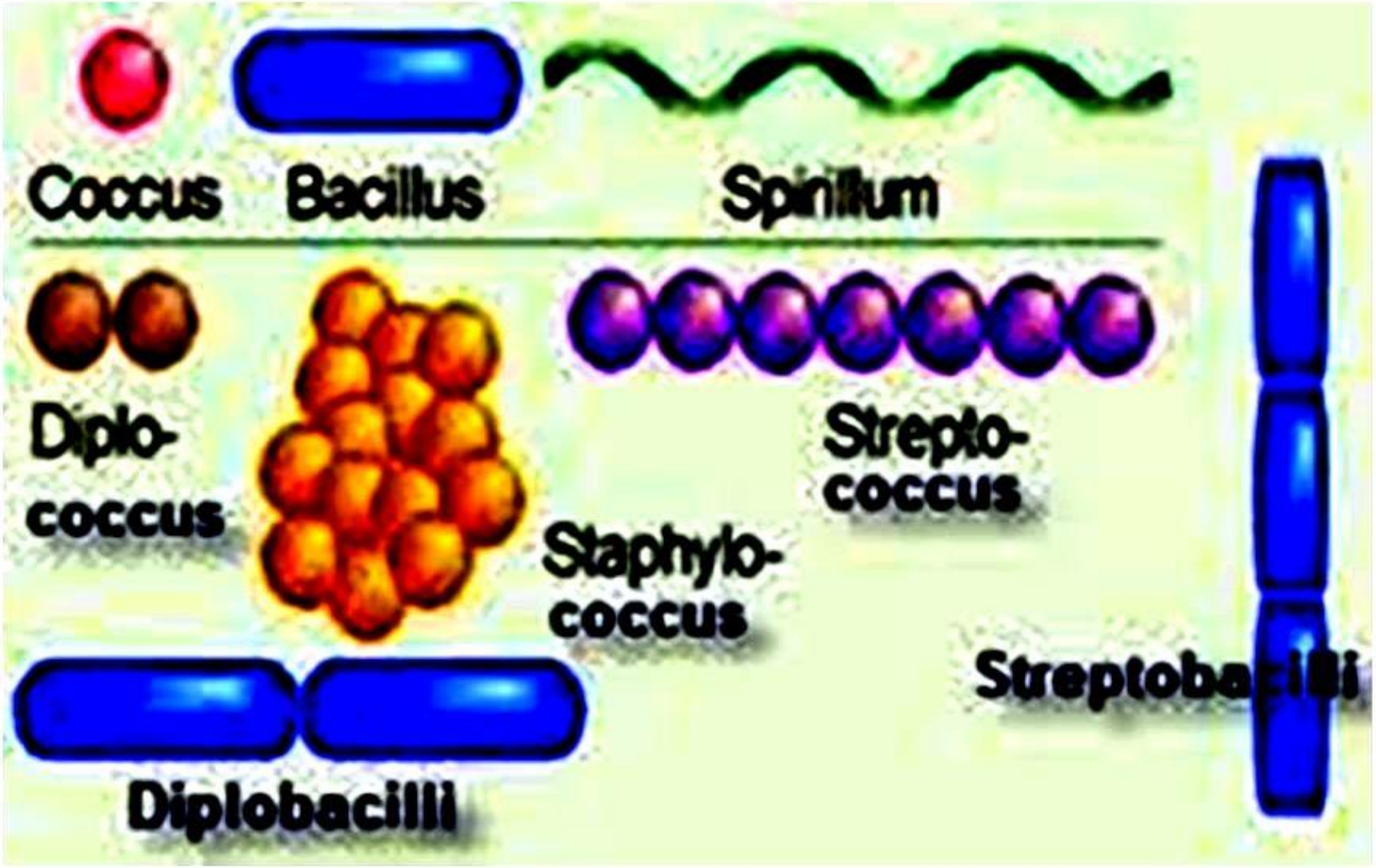
Morphology – spherical / rod /spiral.

Arrangement – clusters/chains.

procedure:


Staining loop
Sink or suitable receptacle
Stain
Staining bottle
(a)
Water
Wash bottle
(b)
Gentle blotting
(c)

Bacterial Shapes and Arrangements



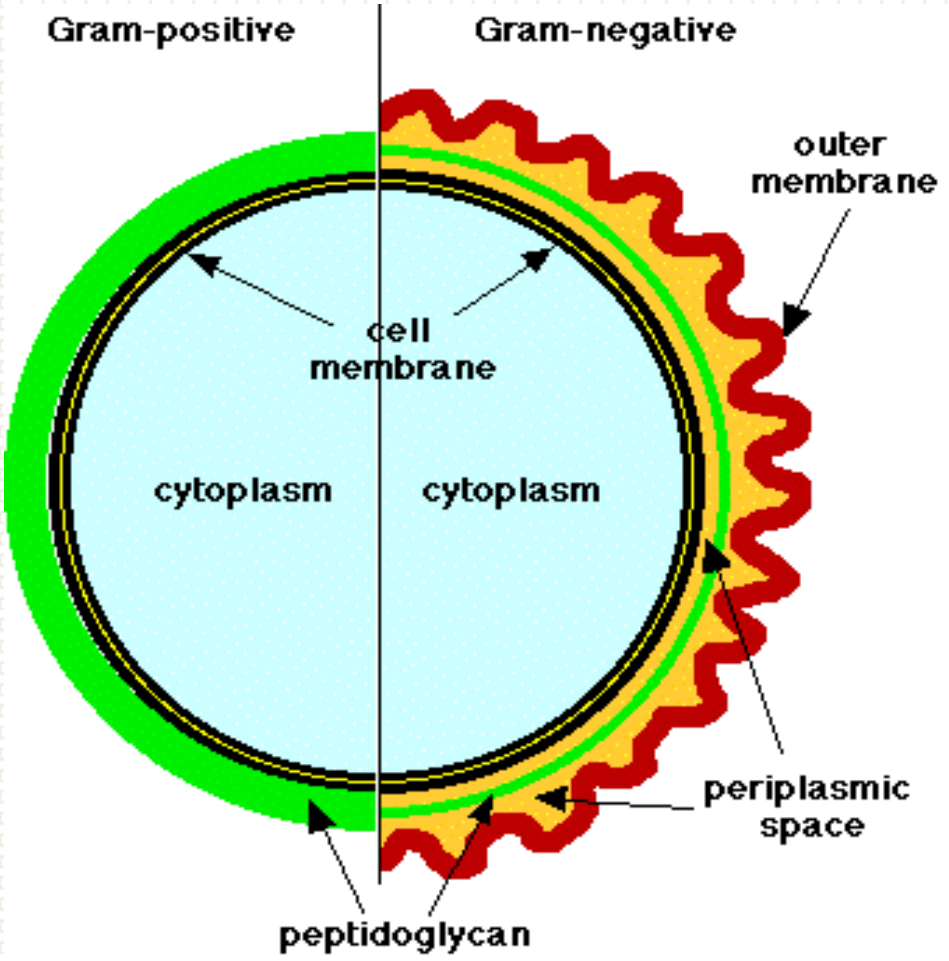
Differential stains

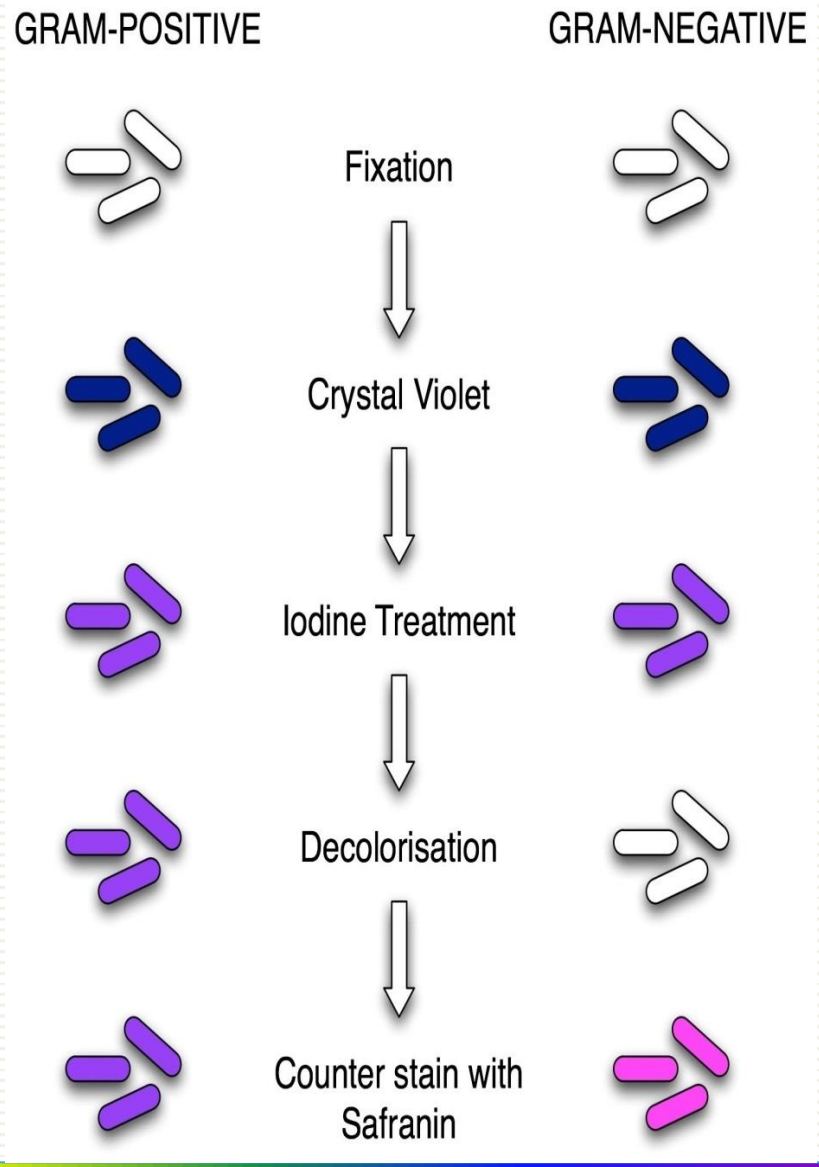
Differential stains, such as the Gram stain and acid-fast stain differentiate bacteria based on the chemical composition of their cell wall. Differential stains use two stains instead of one. The first stain is called the primary stain, and the second is called the counter stain. A decolorization step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

Gram Staining :

- Most bacteria posses 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 retain ether primary stain (G+) or the counter stain (G-) depend on wall composition.

structure and reactivity to gram stain

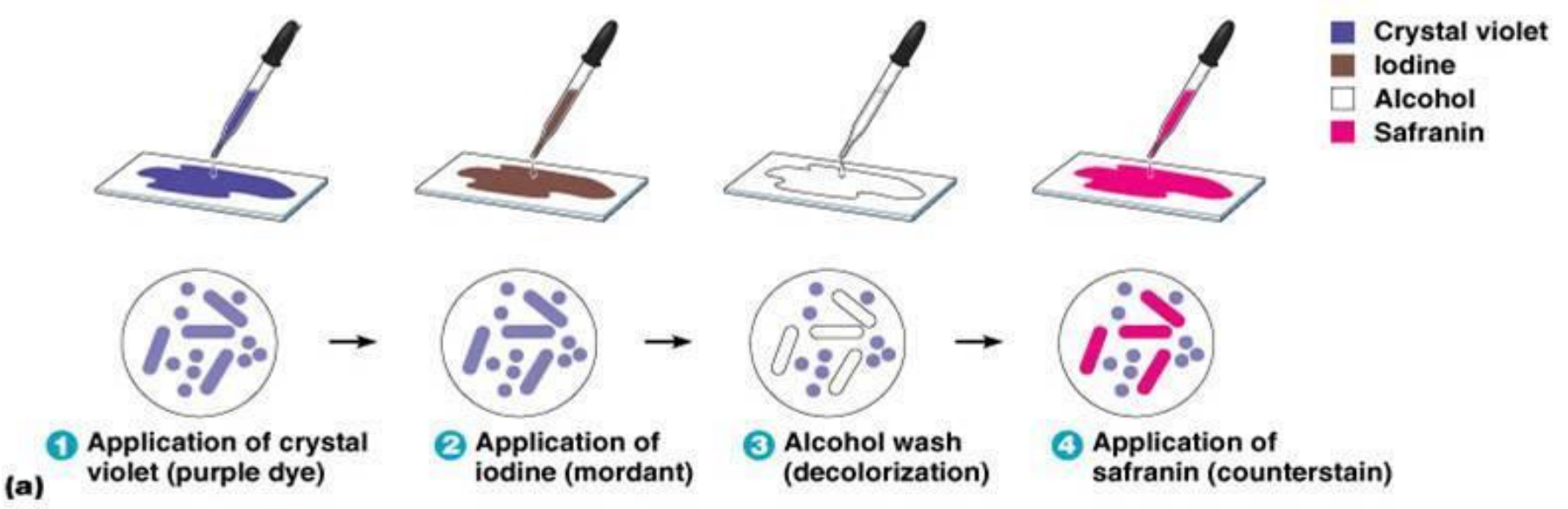




Requirements – Staining Reagents:

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

Procedure of Gram-Stain Technique

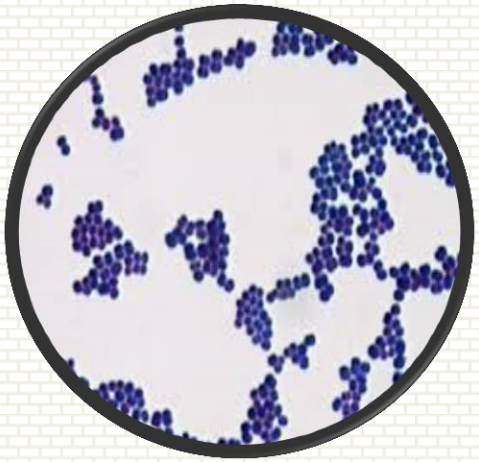


Time Frame

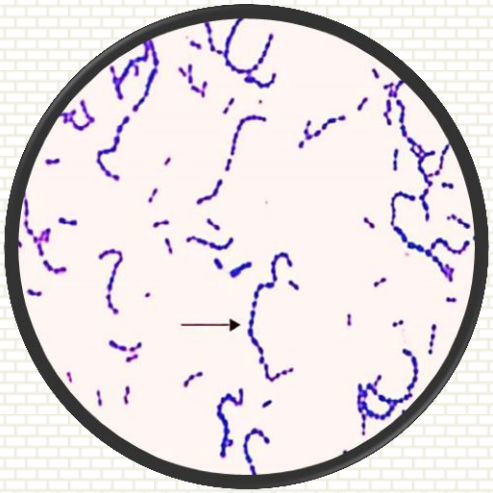
1) 1 minute
2) 1 minute
3) 15 seconds
4) 1 minute

- Rinse with water between each step

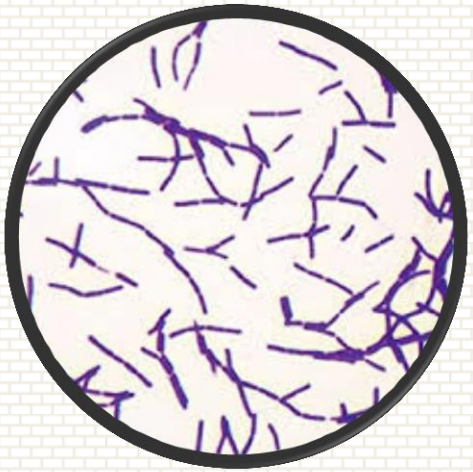
Examples of Gram Positive Bacteria



Gram positive cocci in clusters: Staphylococci

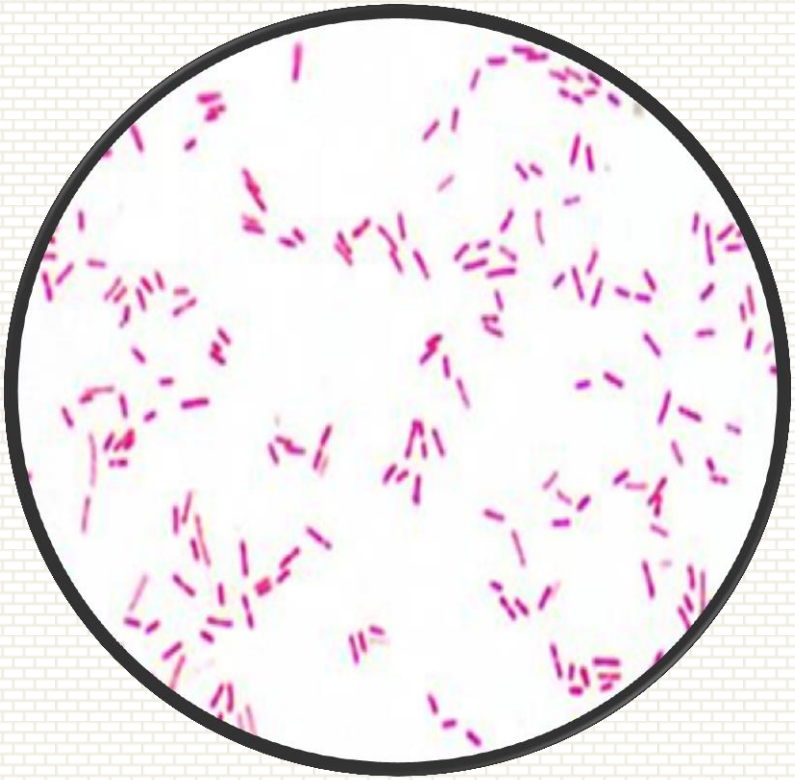


Gram positive cocci in chains: Streptococci

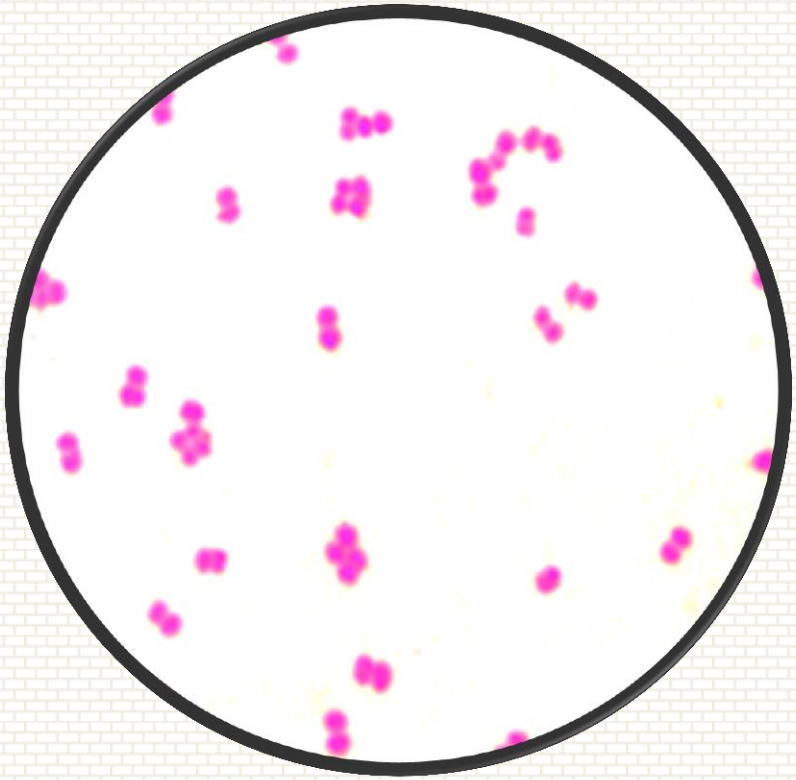


Gram positive bacilli in chains: Bacillus anthracis

Examples of Gram Negative Bacteria



Gram negative bacilli: Escherichia coli



Gram negative cocci: Neisseria species

Acid-fast Staining: (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 this 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:

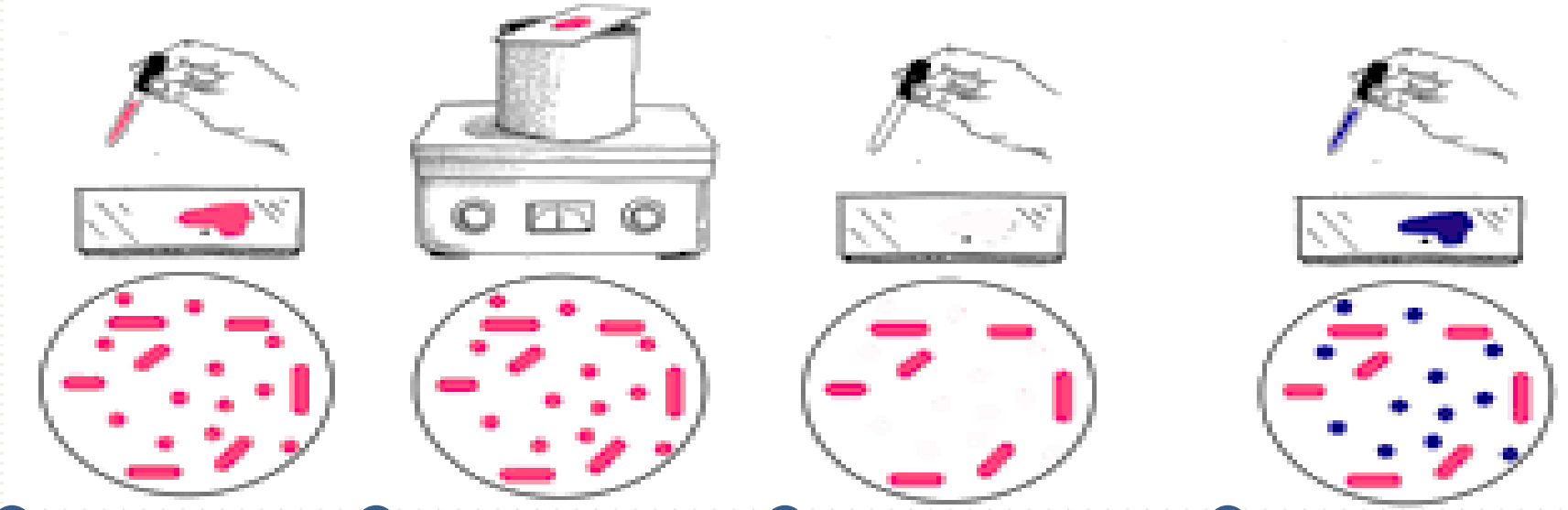
5% sulphuric acid – for M.leprae.

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.

Procedure:



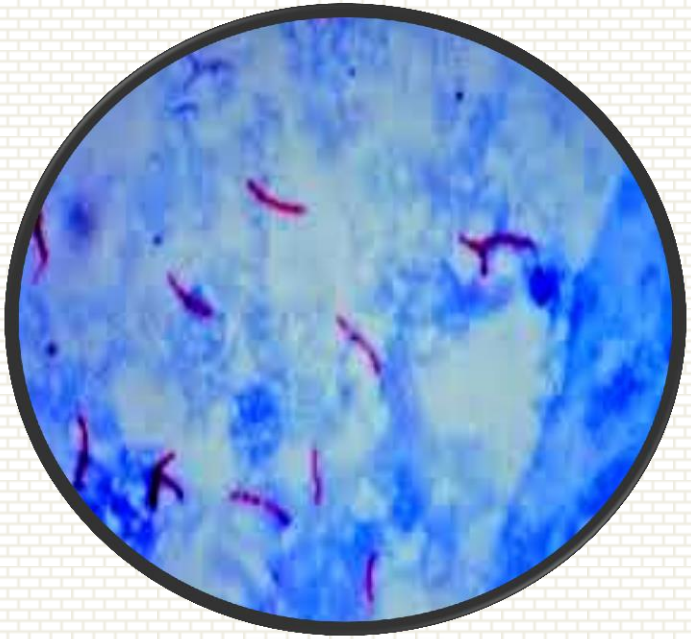
1. Application of carbol fuchsin (primary stain)
2. Application of heat (mordant)
3. Application of acid alcohol (decolorizer)
4. Application of methylene blue (counter stain)

Time Frame

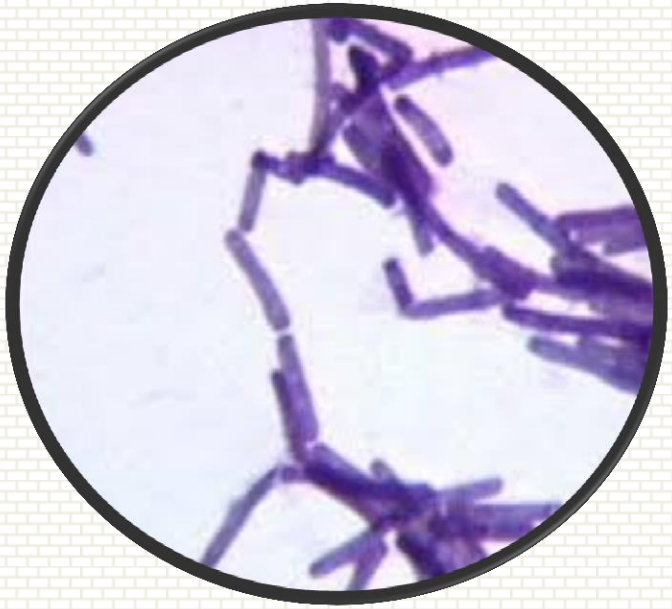
1+2) 5-10 minutes
3) 3-5 minutes
4) 2 minutes

- Rinse with water between each step

Examples of Acid fast and Non-acid fast bacteria



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.

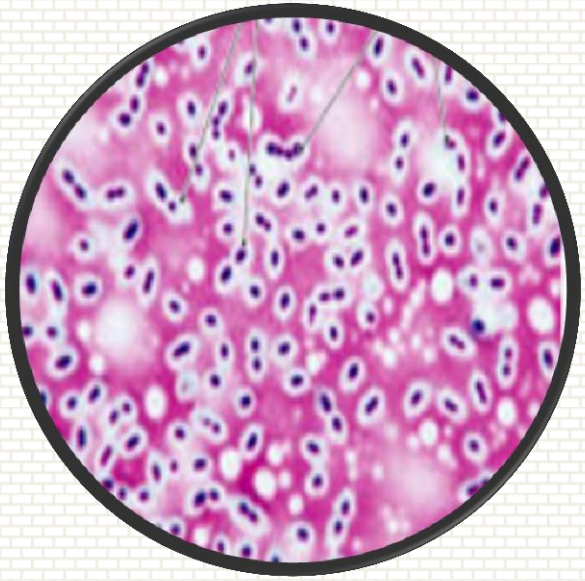
- **Examples of Special Stain:**

1) Capsule Stain - Nigrosin ink+ indian ink

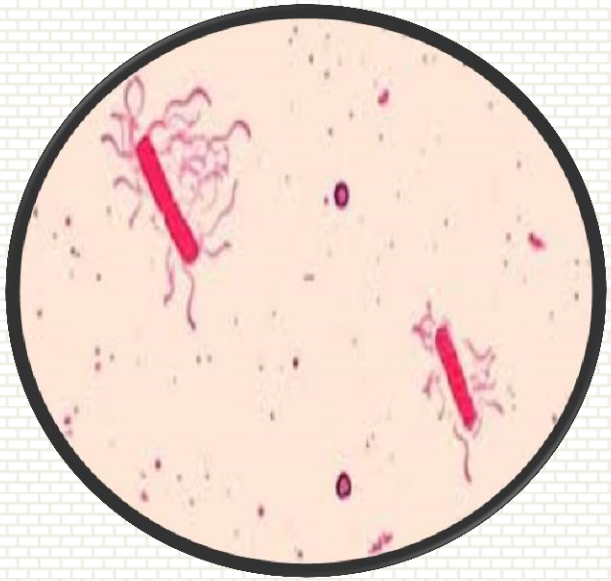
2) Spores - malachite green + safranin

3) Flagella - RYU stain

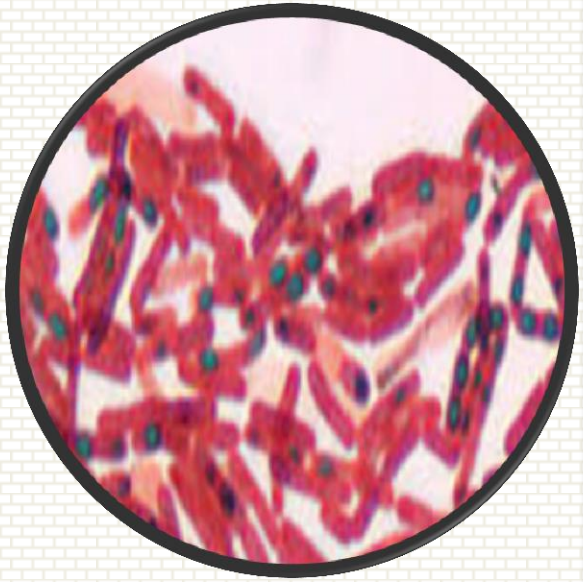
Examples of Special Stain



Capsule Stain



Flagella stain



Spores stain


EVERY ENDING IS
REALLY JUST A
NEW BEGINNING