GRAM'S STAINING

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INTRODUCTION

- The Gram stain was developed in 1884 by the Danish bacteriologist Hans Gram stain .
- It is a very important **differential staining** because it separated bacteria into two broad categories namely **garm positive** and **gram positive .**

History

Hans Christian Joachim Gram

- The Gram stain was devised by the Danish physician, **Hans Christian Joachim Gram,** while working in Berlin in 1883. He later **published this procedure** in 1884. At the time, Dr. Gram was studying lung tissue sections from patients who had died of **pneumonia.**

FOUR BASIC STEPS OF GRAM STAINING

- Applying a primary stain (Crystal Violet) to a heat – fixed smear of a bacterial culture .
- The addition of Gram's iodine , which binds to Crystal Violet and traps it in the cell.
- Decolonization with ALCOHOL or **ACETONE** and,
- Couter staining with safranin.

PRINCIPALE OF GRAM'S STAINING

- Several therories have been proposed to explain the mechanism of Gram's staining, however the one based on **physicochemical nature of the cell wall** of bacteria is widely accepted .
- Cell wall of gram – negative bacteria are generally thinner than those of gram – positive bacteria.
- **Gram- negative bacteria possess higher percentage of lipids** in their cell wall as compared to gram- positive bacteria . During staining the primary stain Crystal Violet forms complex with mordant iodine (CV – I) in the cell wall.

Continue…

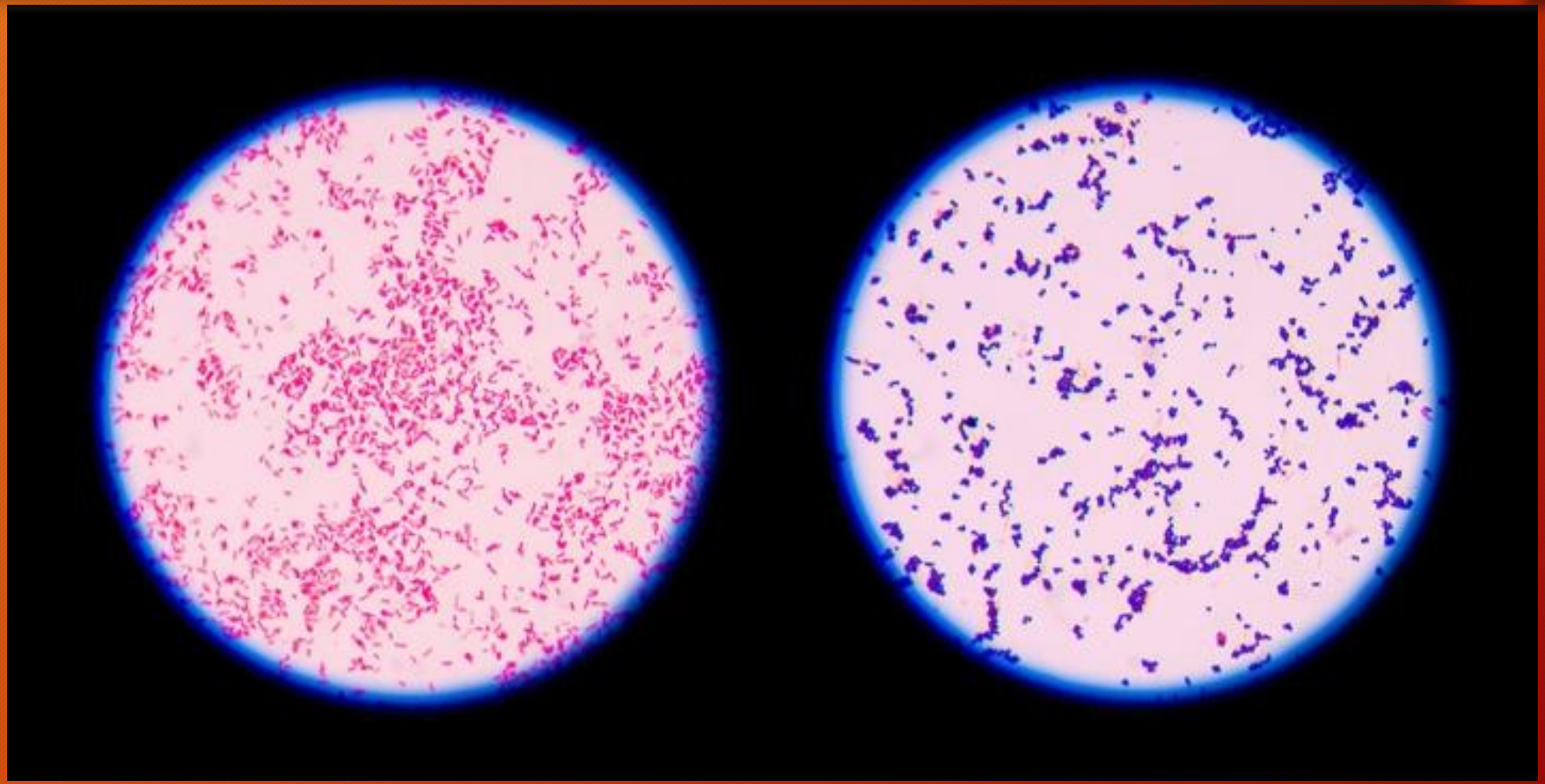
- When gram – positive bacteria are decolonized with ethanol the alcohol is thought to shrink the pores of the thick peptidoglycan.
- Thus , the dye iodine complex is retained during the Short decolonization step and the bacteria remain Violet . In contrast , gram – negative peptidoglycan is very thin , not as highly cross – linked and has larger pores .
- Alcohol treatment also may extract enough lipid from the gram – negative wall to increase its porosity further . For these reasons alcohol more readily remove the Crystal Violet – iodine complex from gram – negative bacteria.
- These cells subsequently take on the color of counterstain the safranin .

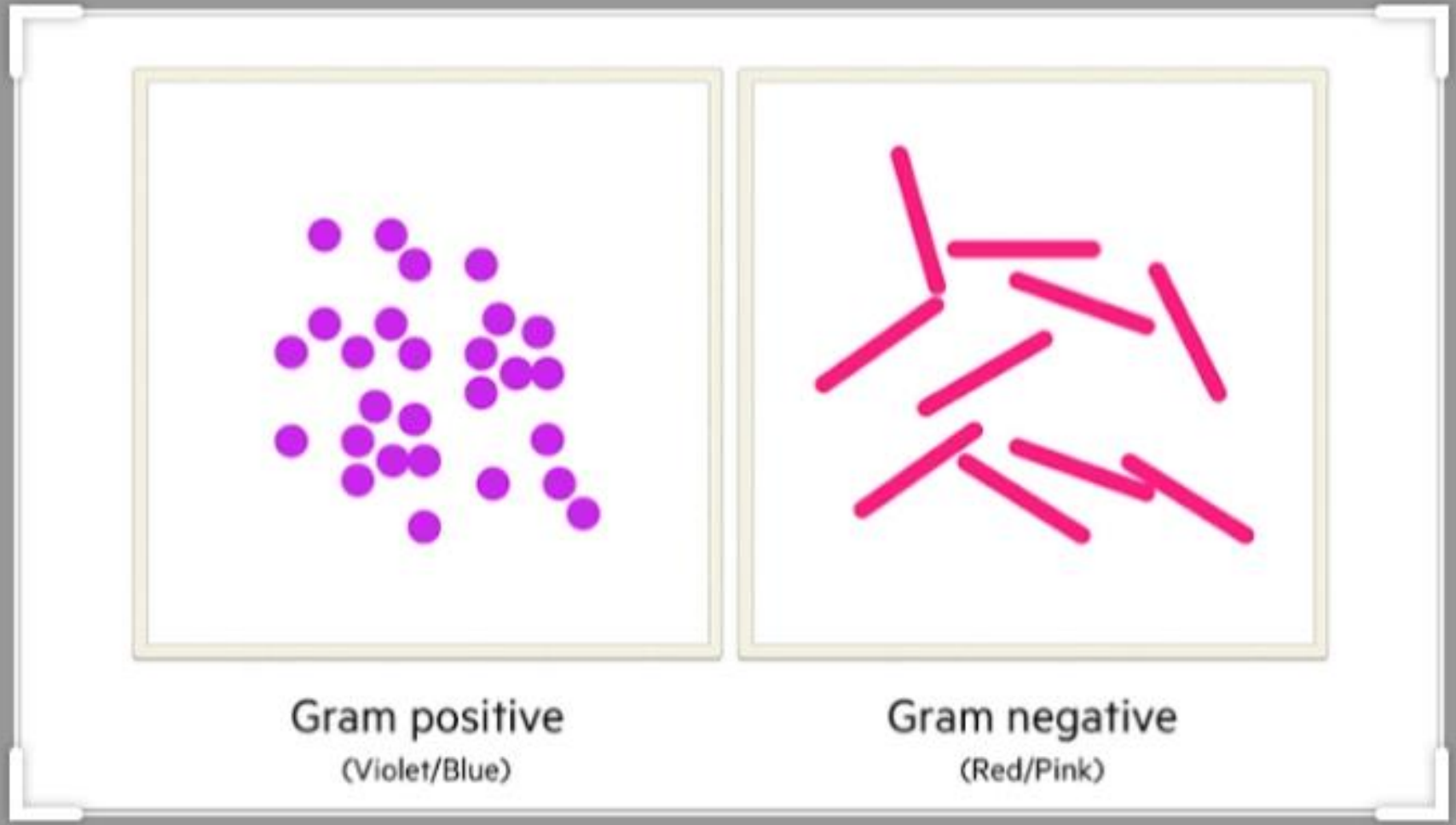
REQUIREMENTS

1. Young cultures of **Escherichia coli** , **Bacillus subtilis** & **Staphylococcus aureus**
2. Crystal Violet stain , Gram's iodine , 95 % ethanol and safranin stain .

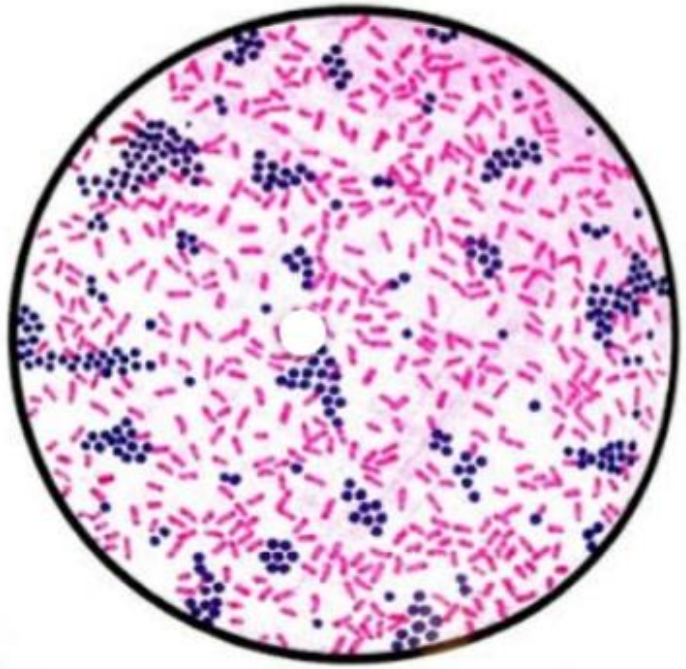
PROCEDURE (HUCKER'S MODIFICATIONS)

1. Prepare a heat fixed smear of the culture.
2. Cover the smear with Crystal Violet stain for 1 minute;
3. Add Gram's iodine to wash off Crystal Violet stain and cover it with iodine till the smear turns coffee brown in color (approximately 1 minute)
4. Rinse the slide in running water.
5. Add decolonizing solution drop wise at the upper end of slide held in inclined position , till the Violet color fails to come out from the smear ; for normal smear 10- 15 seconds are enough
6. Rinse the smear with water.
7. Counterstain with safranin for 45 – 60 seconds.
8. Rinse with tap water, drain, blot , air dry and examine .




Gram positive
(Violet/Blue)
Gram negative
(Red/Pink)

A gram stained bacterial suspension containing a mixture of Gram negative bacilli, and Gram positive cocci arranged in bunches (Staphylococci spp)



FACTORS AFFECTING GRAM REACTION

1. **Age of bacterial cell :** as the ages , gram – posiy cells tend to lose their ability to retain the primary stain and may appear to be gram variable. (i.e. some cells may appear pink)
2. **Decolonization :** Excessive decolonization results in loss of primary stain, causing gram- positive organisms to appear gram – negative . Similarly insufficient decolonization will not completely remove CV – I complex gram – positive organisms to appear gram- positive .
3. **Excessive fixation :** of smear leads to loss in gram positiveness.
4. **pH :** of the culture medium also influence the gram - reaction.
5. **Overcrowding :** of cells in the smear affect the result due to improper decolonization .

EXAMPLE OF GRAM'S STAINING BACTERIA

- GRAM'S POSITIVE BACTERIA

- Bacillus subtilis
- Bacillus megaterium
- Micrococcus luteus
- Staphylococcus aureus
- Micrococcus luteus

- GRAM'S NEGATIVE BACTERIA

- Escherichia coli
- Azotobacter spp.
- Salmonella typhosa
- Methylococcus spp.
- Acidaminococcus spp.