

# PRACTICAL

## GRAM STAINING

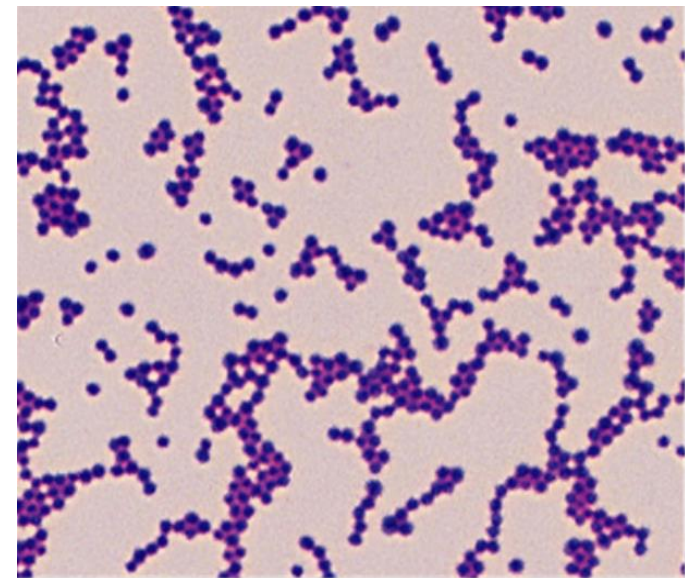
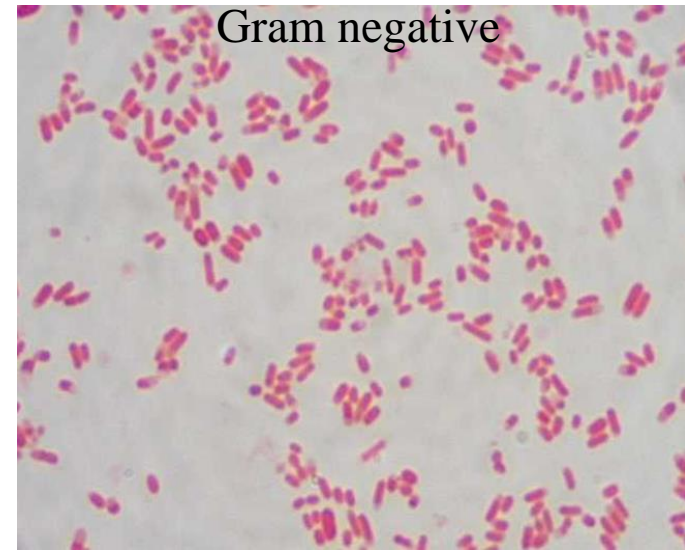
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# OBJECTIVES

- Describe reagents used in Gram stain & purpose of these reagents
- Color expected of Gram Positive & Negative after performing the procedure
- Explain reason of differential stain by Gram Positive & Negative
- Describe cell wall structure of Gram Positive & Negative
- Explain Gram variable organism

# REAGENTS USED IN GRAM STAIN

1. Gram Crystal Violet 0.5%
2. Gram Iodine
  - a. Potassium Iodide 2%
  - b. Resublimed Iodine 1%
3. Gram Decolourizer
  - a. Methanol 80%
  - b. Acetone 20%
4. Gram Safranin 1%



Gram positive

# REAGENTS USED IN GRAM STAIN

## 1. CRYSTAL VIOLET

- Primary stain
- Violet colored, stains all micro-org

## 2. GRAM IODINE

- Mordant
- Forms Crystal violet iodine complexes

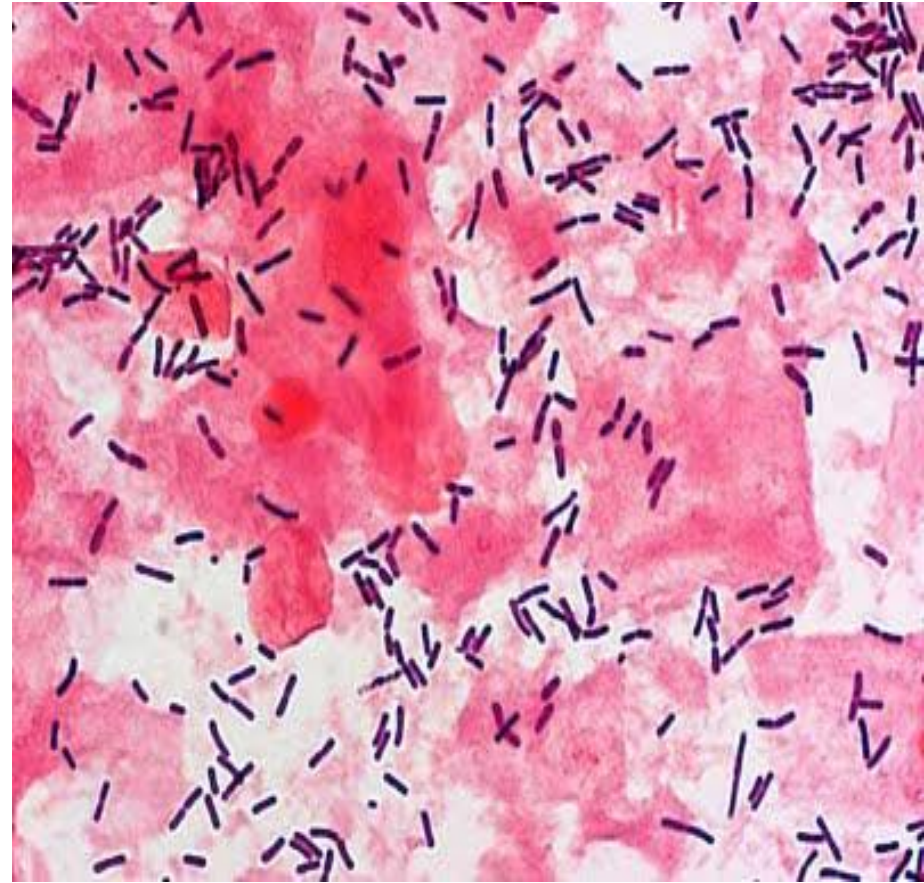
## 3. DECOLORIZER

- Acetone + Methanol
- Removes Crystal violet iodine complex from thin peptidoglycan layers
- Dissolves outer layer of Gram negative org

# REAGENTS USED IN GRAM STAIN

## 4. GRAM SAFRANINE

- Counter stain
- Red colored
- Stains thin walled Gram negative organism
- Pus cells cytoplasm & lobes of nuclei also stain red



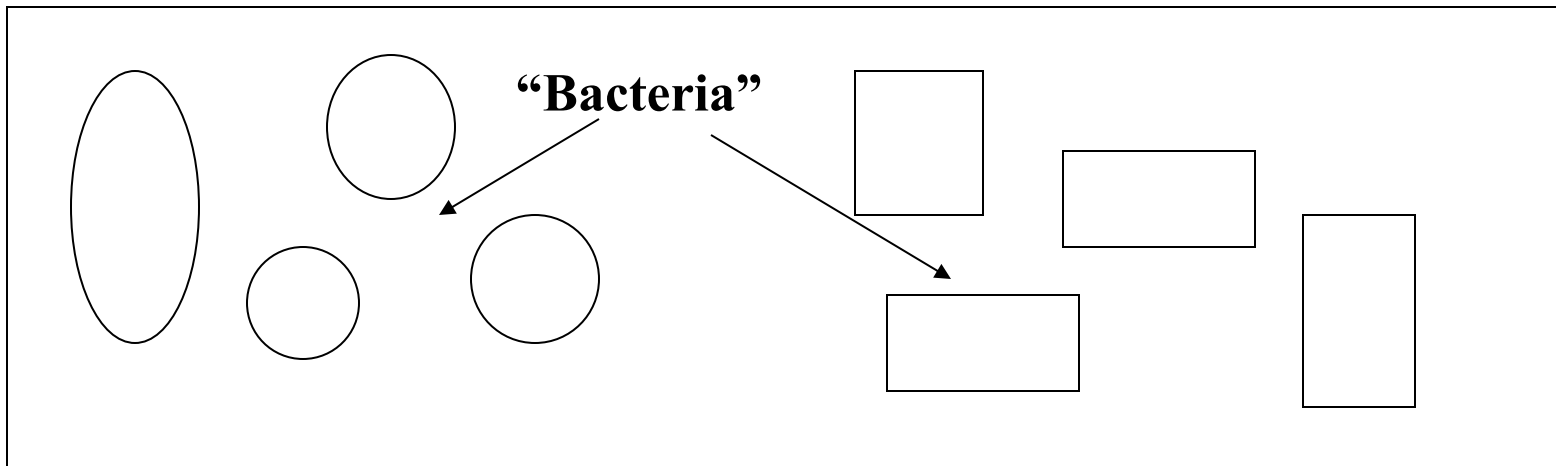
# THE GRAM STAIN PROCEDURE

## Step 1 - Prepare a Smear

Suspend some of the material to be stained in a \_\_\_\_\_ drop of water on a microscope slide, spread the \_\_\_\_\_ drop to about the size of a nickel.

Allow to air dry. Heat fix by gently warming

**Watch what happens to the “Bacteria” at each step**



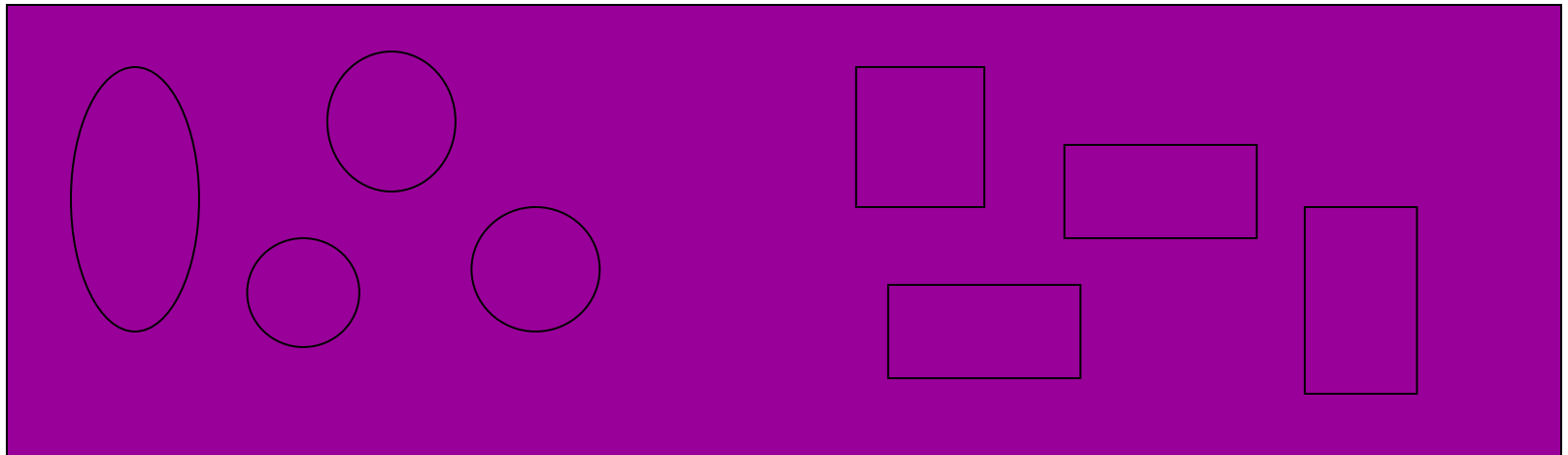
# THE GRAM STAIN PROCEDURE

## Step 2 - Apply the Primary Stain

Flood the Smear with **Crystal Violet**

Allow to stand for 1 min

Rinse with water to remove excess stain

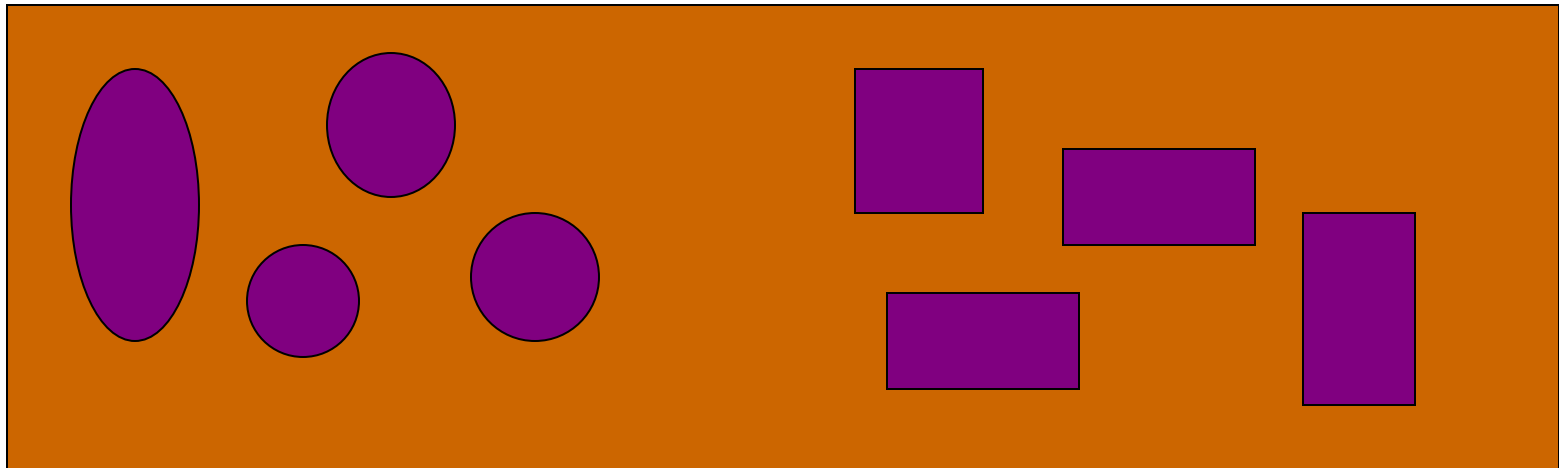


# THE GRAM STAIN PROCEDURE

## Step 3 - Apply the Mordant

Flood the Smear with **Iodine** solution

Allow to stand 2 min

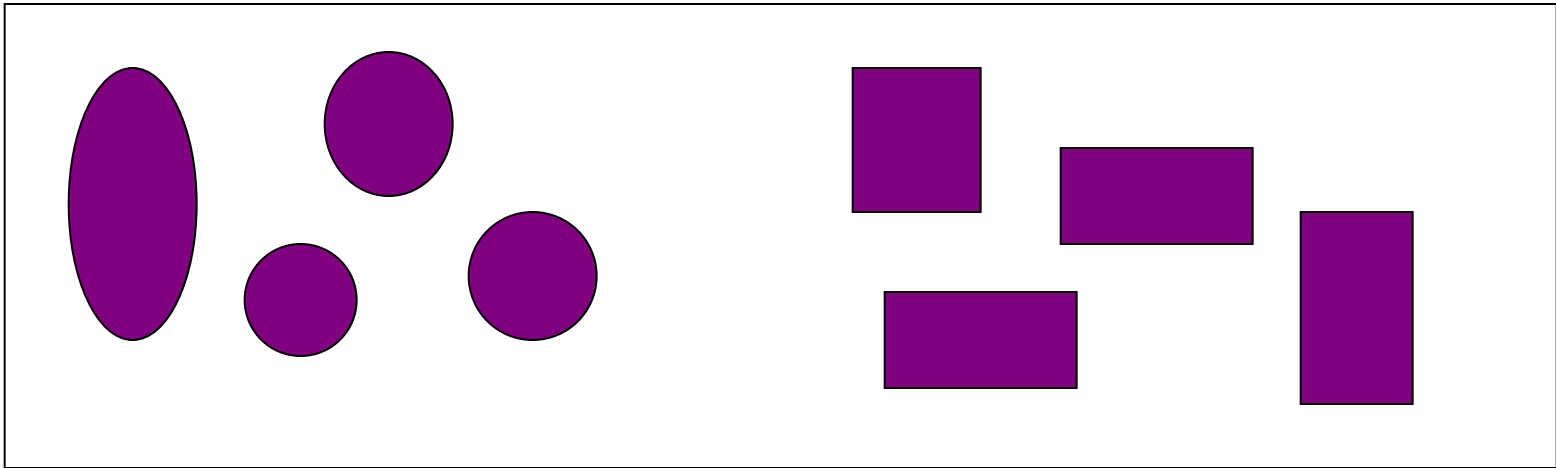




# THE GRAM STAIN PROCEDURE

## Step 4 - Rinse

Rinse with water to remove excess Iodine

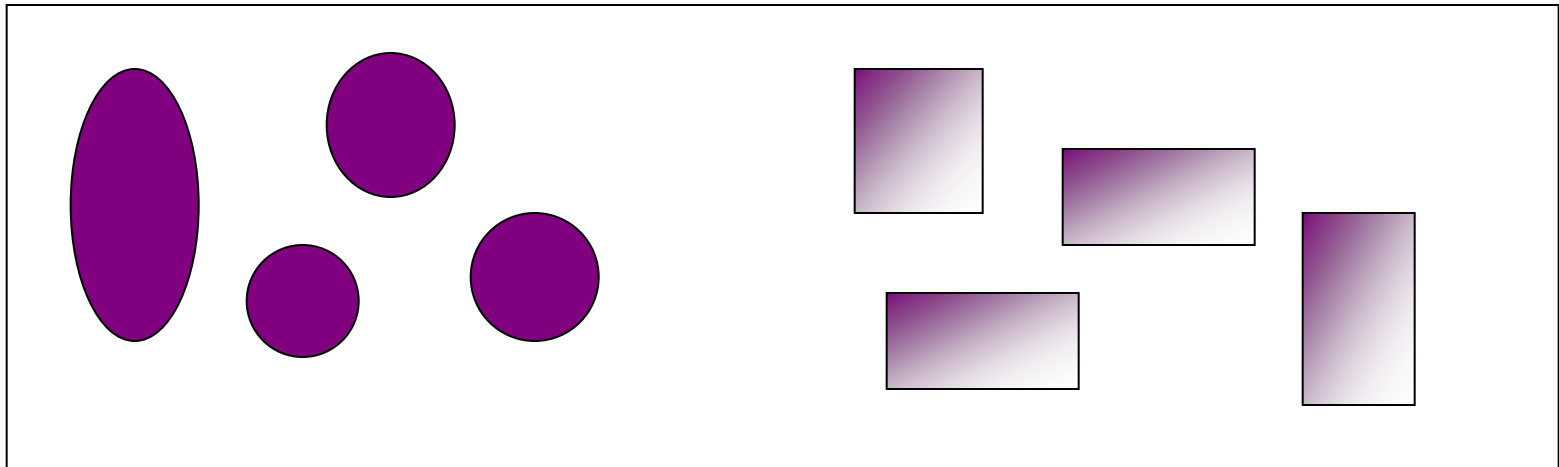


# THE GRAM STAIN PROCEDURE

## Step 5 - Decolorize

Drip Decolorizer (80% Methanol +20% Acetone) across the slide about 5 sec

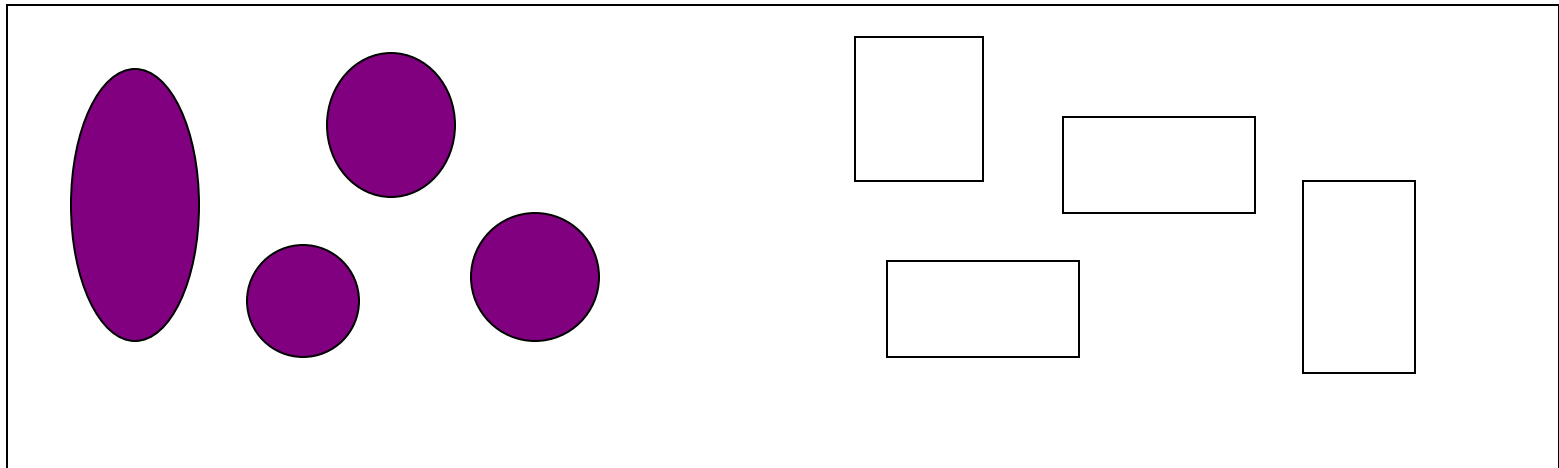
The effluent should appear pale or clear



# THE GRAM STAIN PROCEDURE

## Step 6 - Rinse

Rinse with water to remove excess alcohol

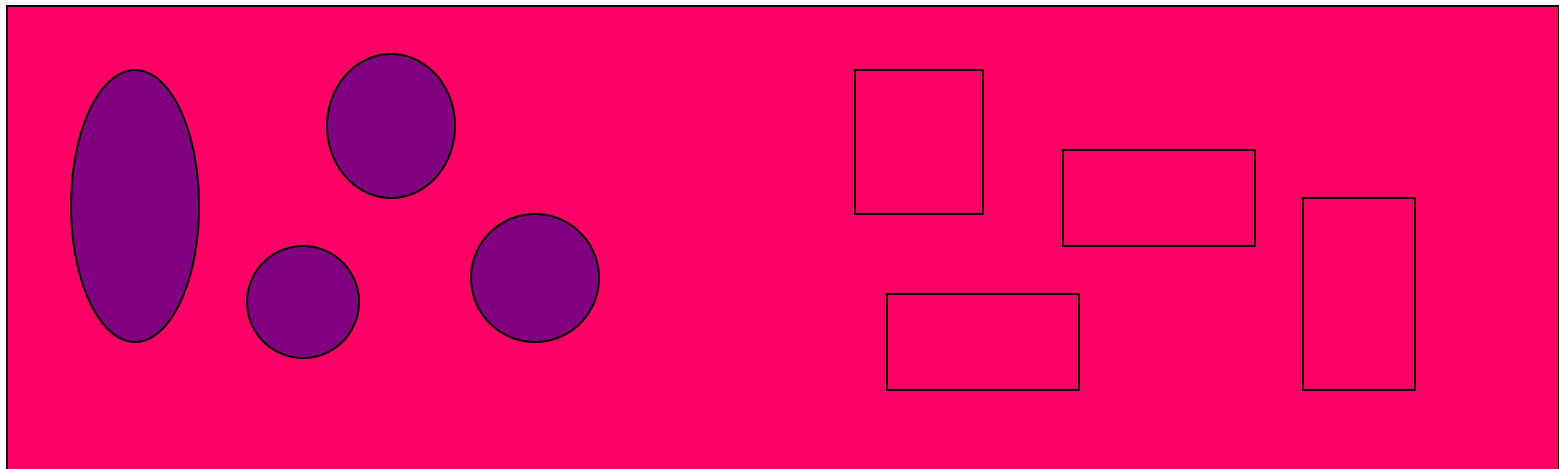


# THE GRAM STAIN PROCEDURE

## Step 7 - Counterstain

Flood the slide with **Safranin** solution

Let stand for 2 minutes



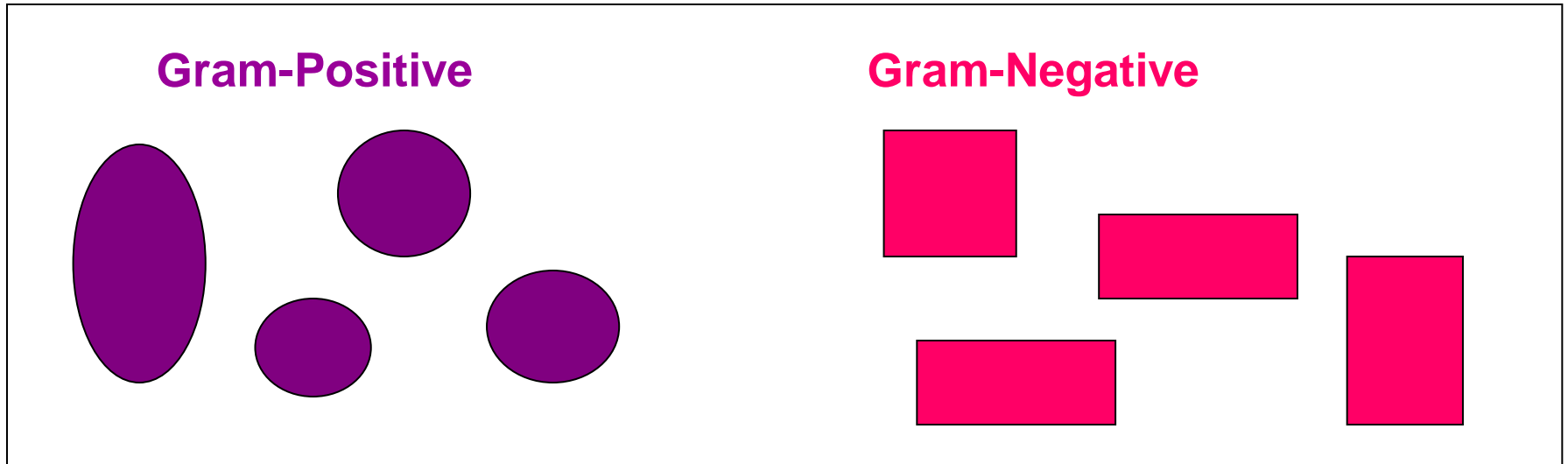
# THE GRAM STAIN

## Step 8 - Rinse, Dry and Observe

Rinse with water to remove excess stain

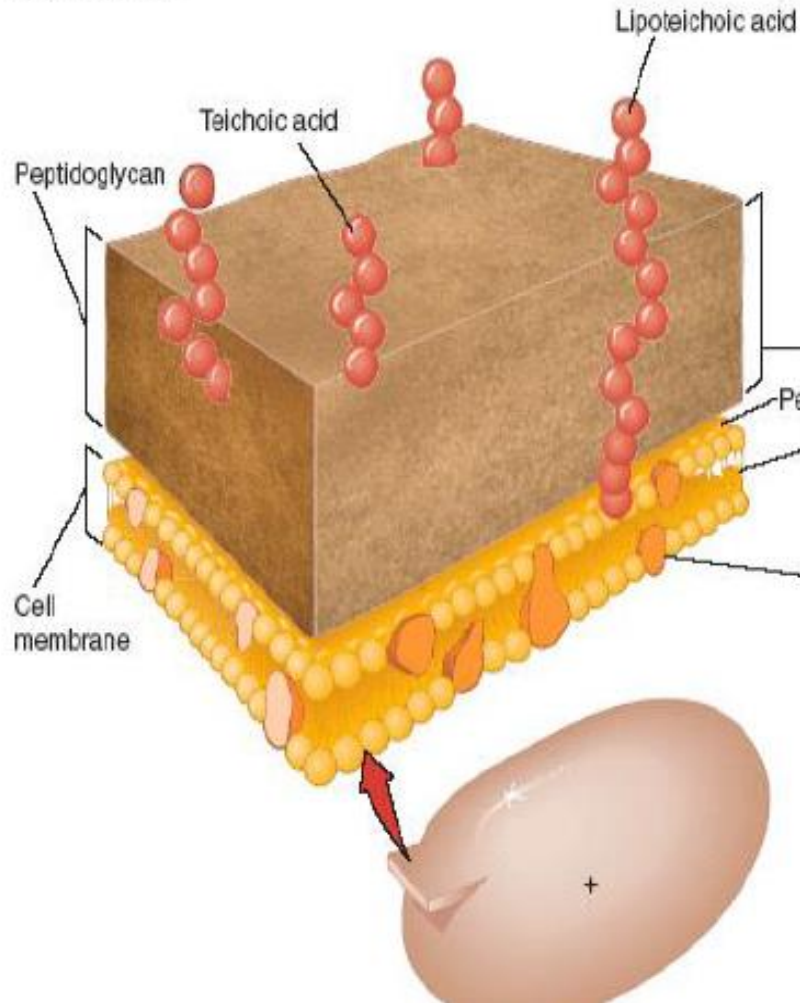
Blot dry

Observe under Oil Immersion

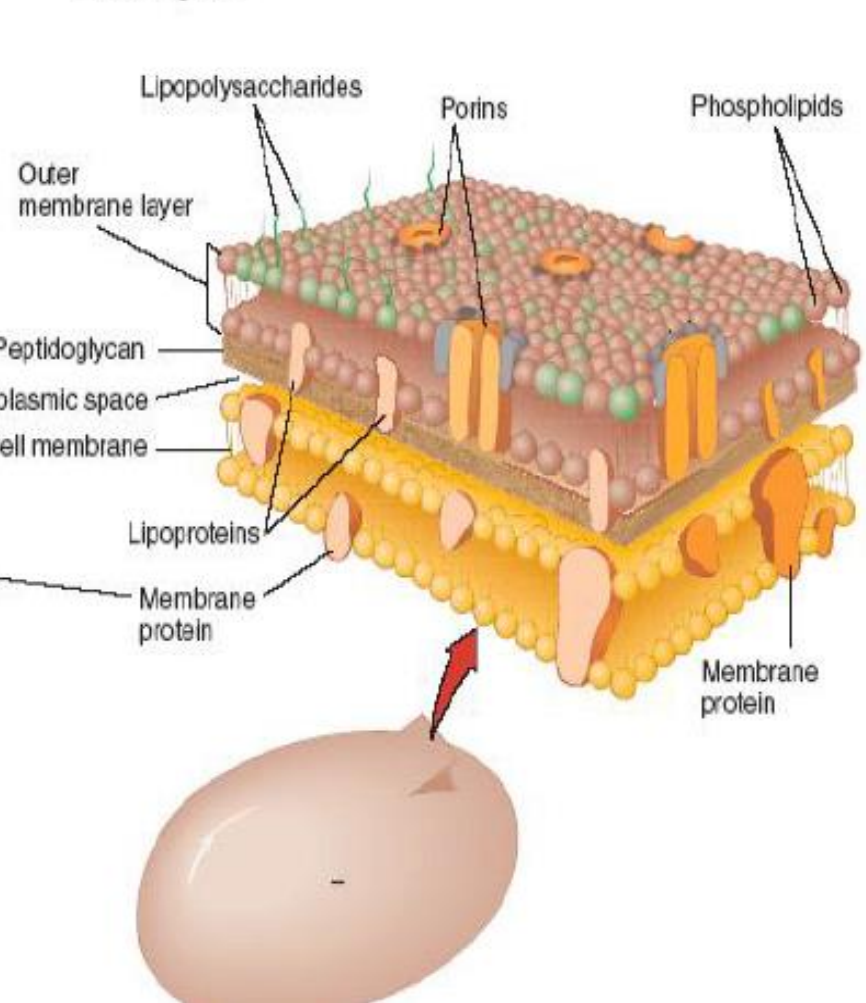


# CELL WALL OF GRAM POS & NEG

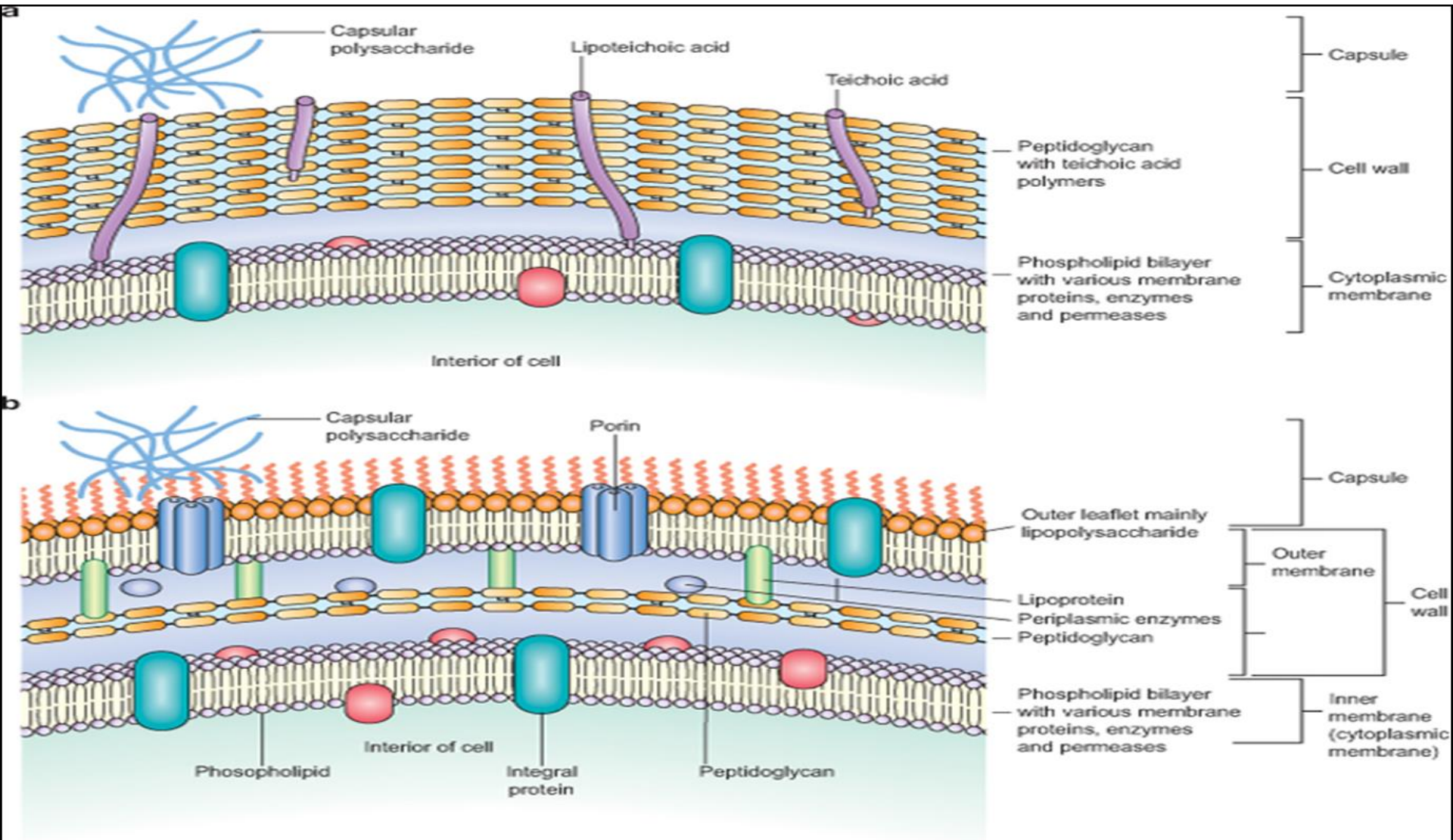
Gram Positive



Gram Negative



# CELL WALL OF GRAM POS & NEG



# CELL WALL IN GRAM +VE AND GRAM -VE BACTERIA

Cell Wall Structures	Gram Positive organisms	Gram Negative organisms
Inner cytoplasmic membrane	Present	Present
Peptidoglycan layer	Thick	Thin
Teichoic Acid	Present	Absent
Outer membrane layer	Absent	Present
Lipid A, LPS , Lipoprotien components	Absent	Present
Peri-plasmic space	Absent	Present



# GRAM VARIABLE

- Gram variability
- Old cultures
- Decolorize improperly
- Dead and alive bacteria
- Autolytic organisms  
e.g. *Streptococcus pneumoniae*

