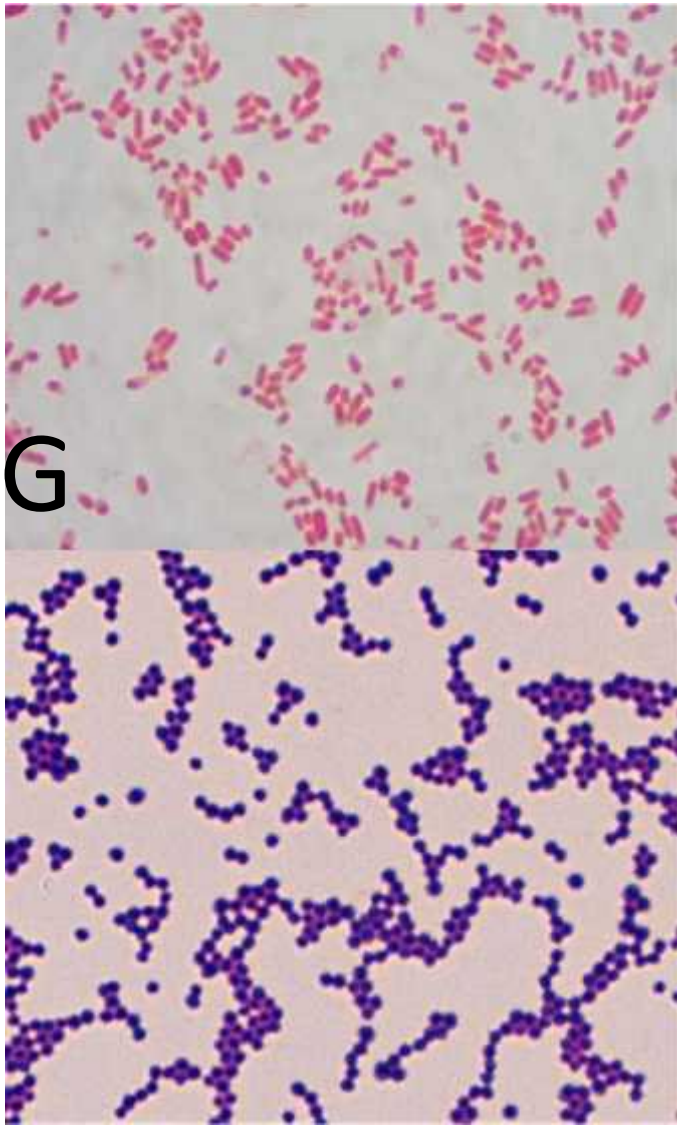
REAGENTS USED IN GRAM STAIN

PRACTICAL
GRAM STAINING



HANAN SAMI
MSC MICROBIOLOGY

REAGENTS USED IN GRAM STAIN

0.5% Gram Crystal Violet 1.

Gram negative

Gram Iodine 2.

2% a. Potassium Iodide

1% b. Resublimed Iodine

Gram Decolorizer 3.

80% a. Methanol

20% b. Acetone

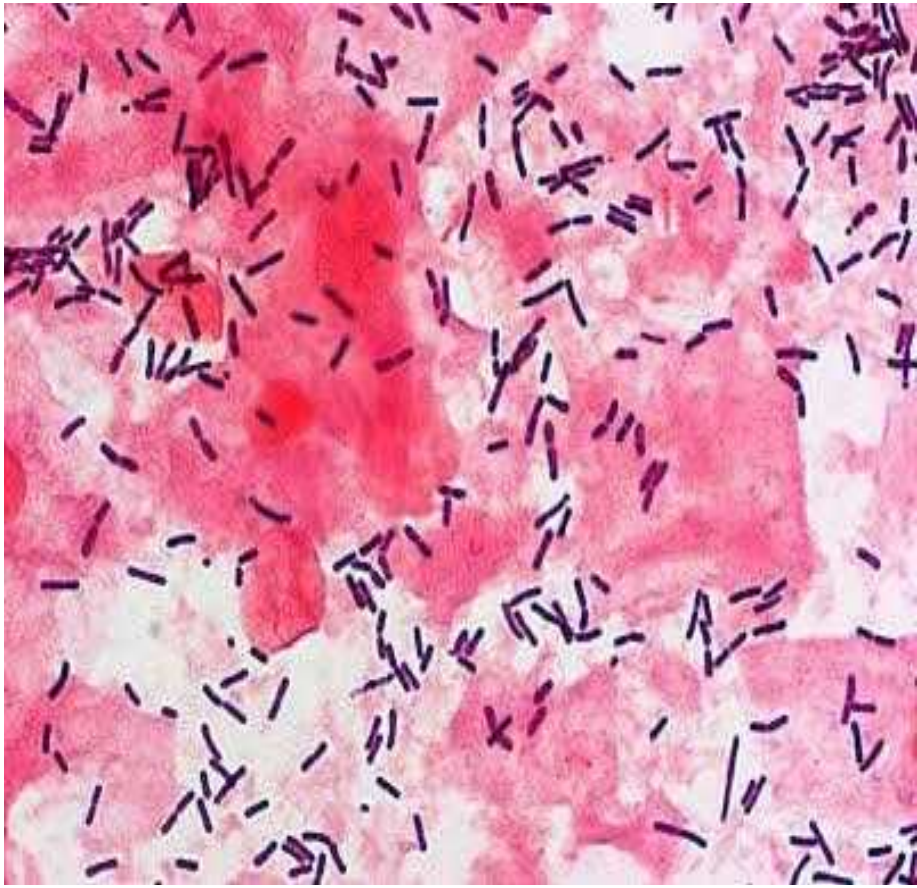
1% Gram Safranine 4.

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 neg org
   - Pus cells cytoplasm & lobes of nuclie also stain red



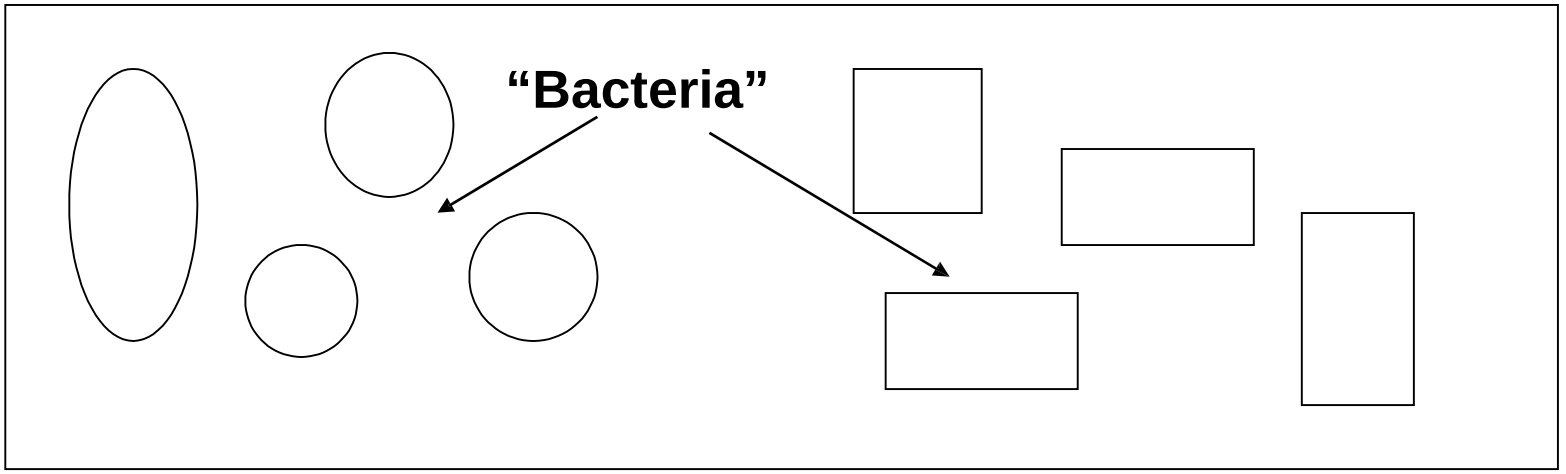
The Gram Stain Procedure

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



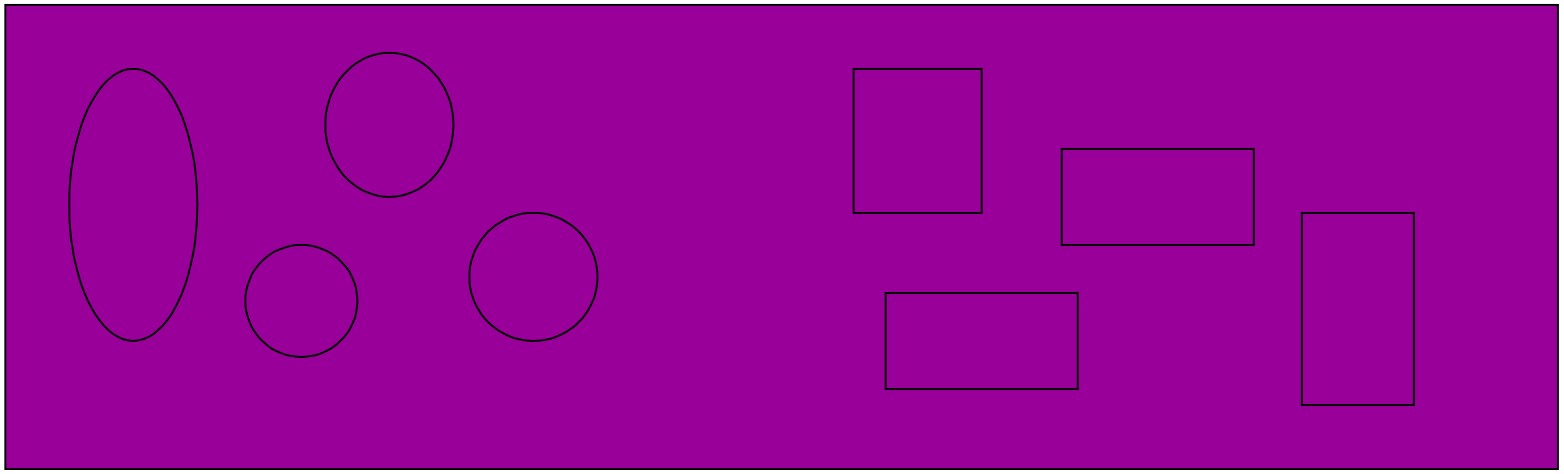
The Gram Stain Procedure

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

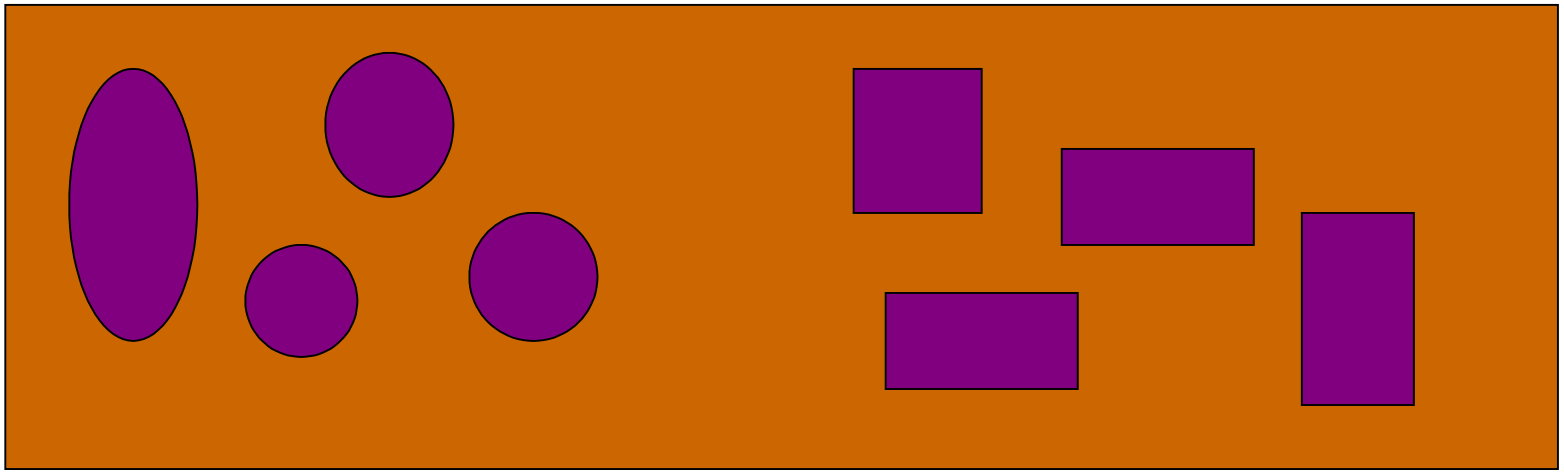


The Gram Stain Procedure

Step 3 - **Apply the Mordant**

Flood the Smear with **Iodine** solution

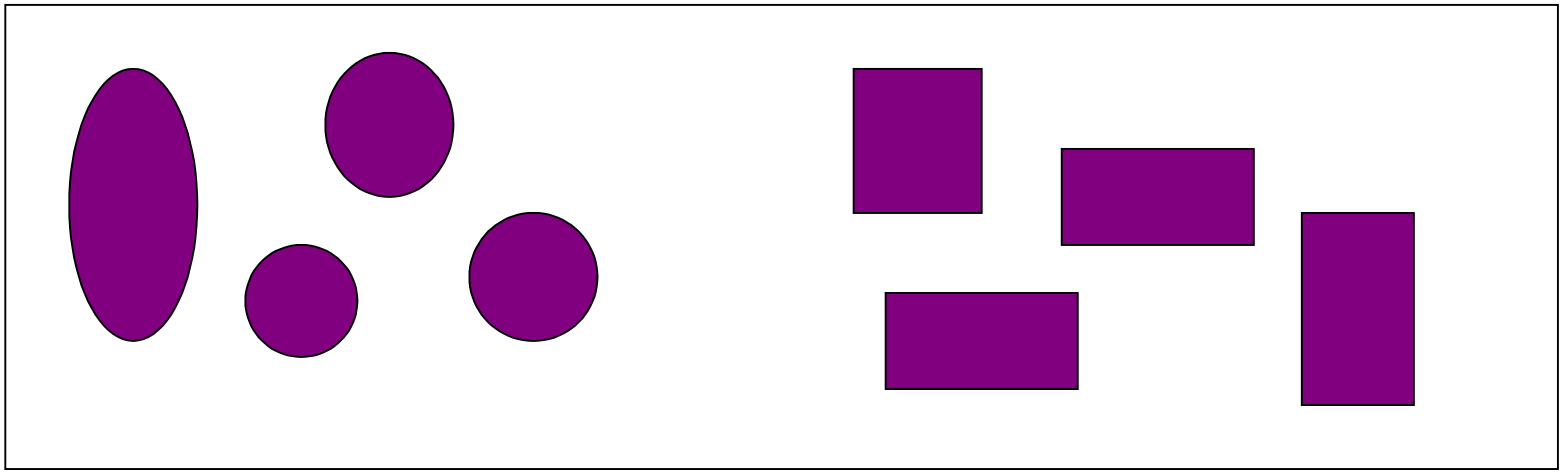
Allow to stand 2 min



The Gram Stain Procedure

Step 4 - **Rinse**

Rinse with water to remove excess Iodine

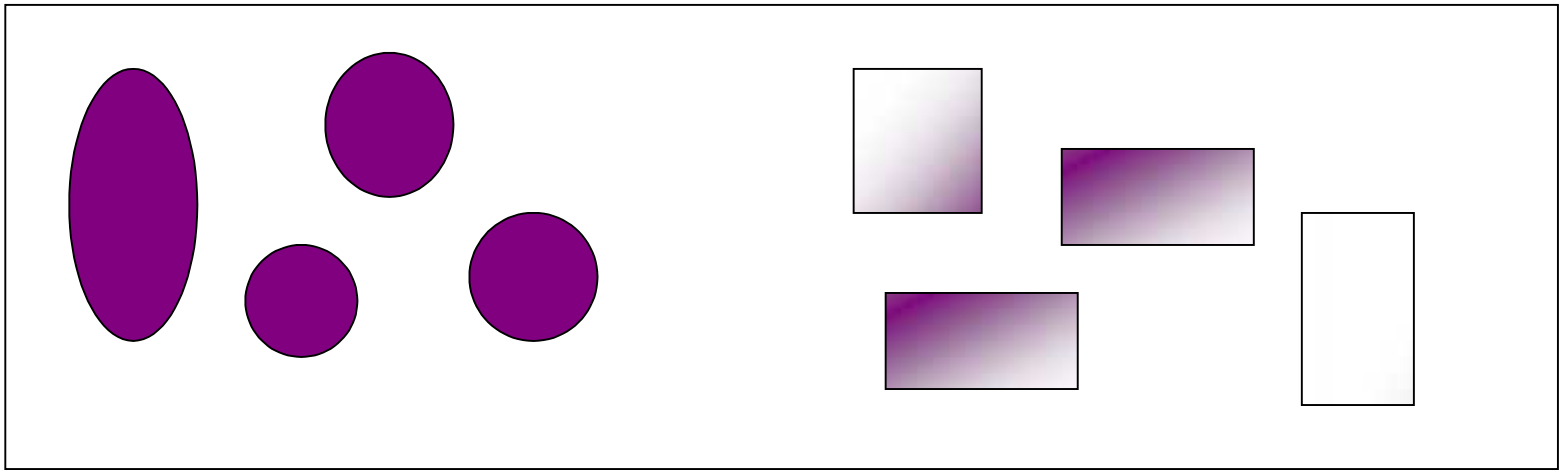


The Gram Stain Procedure

Step 5 - **Decolorize**

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

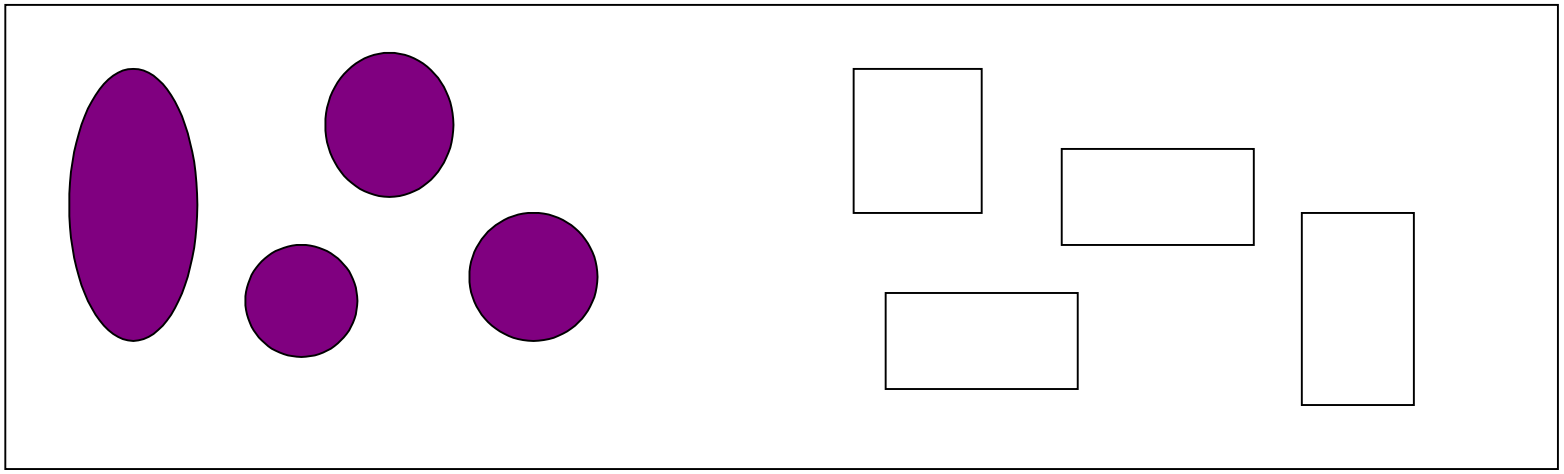
The effluent should appear pale or clear



The Gram Stain Procedure

Step 6 - **Rinse**

Rinse with water to remove excess alcohol

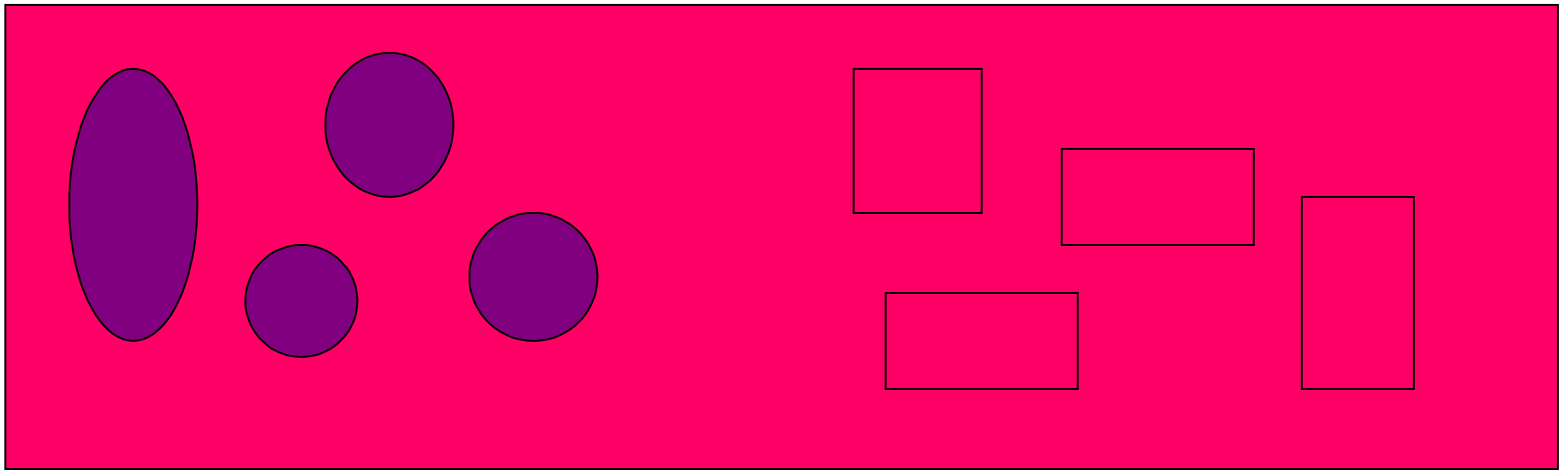


The Gram Stain Procedure

Step 7 - **Counterstain**

Flood the slide with **Safranin** solution

Let stand for 2 minutes



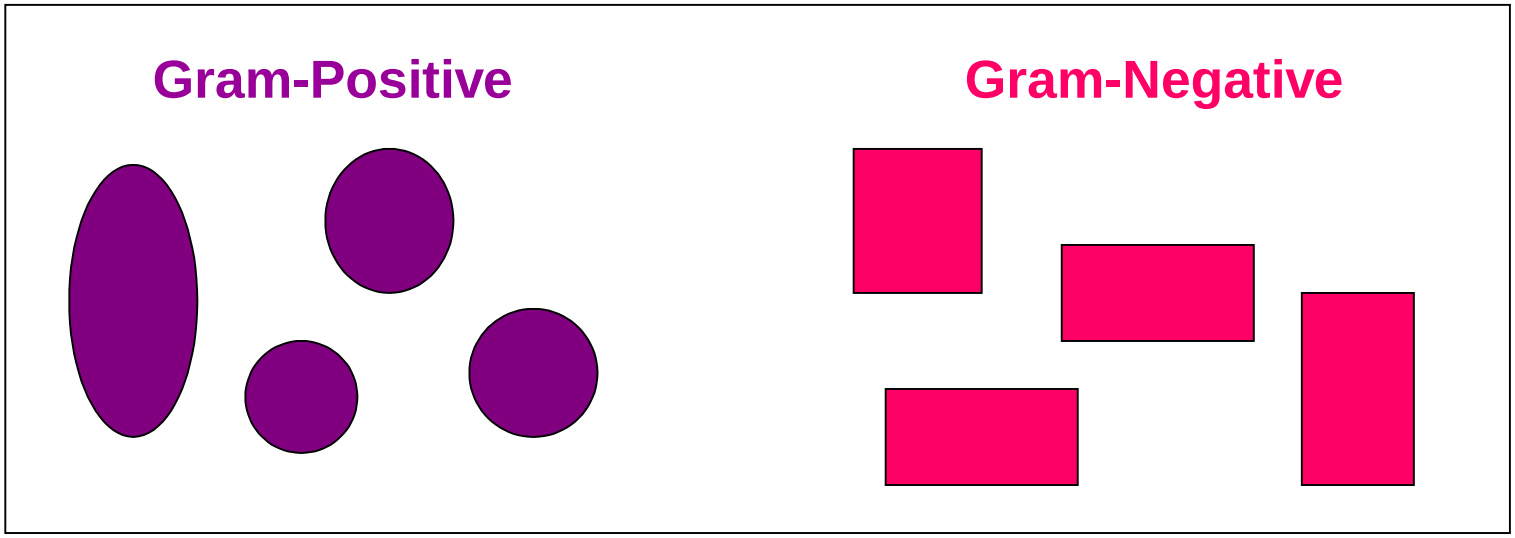
The Gram Stain

Step 8 - **Rinse, Dry and Observe**

Rinse with water to remove excess stain

Blot dry

Observe under Oil Immersion



CELL WALL 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 , Lipo-protien components	Absent	Present
Peri-plasmic space	Absent	Present