LAB 7. GRAM STAIN

The Gram stain is the most commonly used stain in the clinical microbiology laboratory.

It places bacteria into one of two main groups: **gram-positive** (blue to purple) or **gram-negative** (pink)

- The cell wall structure determines the Gram-staining characteristics of a species.

COMPONENTS OF GRAM STAIN

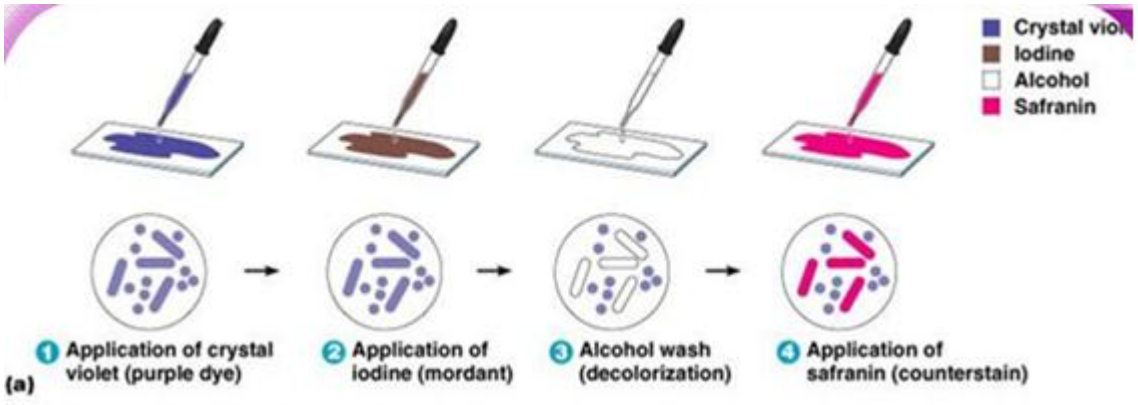
The Gram stain consists of gentle heat fixing (**methyl alcohol** may also be used to fix) of the smear and the addition of **four sequential components**:

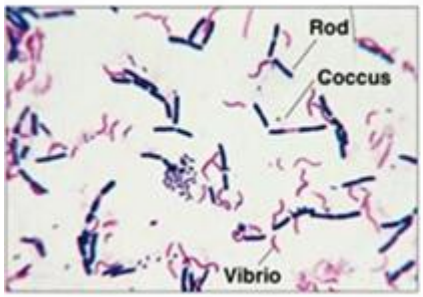
- **crystal violet** (the primary stain, 1 minute)
- **iodine** (the mordant or fixative, 1 minute)
- **alcohol or an alcohol acetone solution** (the decolorizer, quick on and rinse
- **safranin** (the counterstain, 30 seconds).

PROCEDURE

1. Heat-fix specimen to slide. Flood slide with crystal violet solution; allow to act for 1 minute.
2. Rinse the slide, then flood with iodine solution; allow iodine to act for 1 minute. Before acetone decolorization , all organisms appear purple, that is, gram-positive.
3. Rinse off excess iodine. Decolorize with acetone for 5 seconds (time depends on density of specimen).

4. Wash slide immediately in water. After acetone decolorization, those organisms that are gram negative are no longer visible. Apply safranin counter stain for 30 seconds.
5. Apply safranin counter stain for 30 seconds.





❖ *Rinse with water between each step*