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.

