Gram Stain

Dr Murtakab Y Al-Hejjaj

Molecular Biology and Biotechnology

Gram stain

- The most widely used staining procedure in microbiology is the Gram stain, discovered by the Danish scientist and physician Christian Gram in 1884. A staining technique divides bacteria into two groups; gram-positive and gram-negative.

Principles

- Gram staining is based on the ability of bacteria cell wall to retain the crystal violet dye during solvent treatment.
- The cell walls for Gram-positive microorganisms have a higher peptidoglycan and lower lipid content than gram-negative bacteria.
- Bacteria cell walls are stained by the crystal violet. Iodine is subsequently added as a mordant to form the crystal violet-iodine complex, so that the dye cannot be removed easily. This step is commonly referred to as fixing the dye.

- Subsequent treatment with a decolorizer, such as 95% ethanol, **dissolves the lipid layer from the gram-negative cells**.
- The removal of the lipid layer enhances the leaching of the primary stain from the cells into the surrounding solvent. In contrast, the solvent **dehydrates the thicker Gram-positive cell walls**, closing the pores as the cell wall shrinks during dehydration.
- As a result, the diffusion of the violet-iodine complex is blocked, and the bacteria remain stained.

Procedure

•Aseptically, make a smear of the bacterial sample with an inoculating loop, air dry and heat-fix the organism on a glass slide.

•Cover the smear with **crystal violet** and let it stand for 20 seconds.

•Briefly wash off the stain with distilled water. Drain off excess water.

•Cover the smear with **Gram's iodine** solution and let it stand for one minute.

- Pour off the Gram's iodine and flood the smear with **95% ethyl alcohol** for 10 to 20 seconds.
- Stop action of the alcohol by rinsing the slide with water for few seconds.
- Cover the smear with **safranin** for 20 seconds.
- Wash gently for few seconds, blot dry with paper, and air-dry.
- Examine the slide under oil immersion.