Differential Stains

Gram Stain

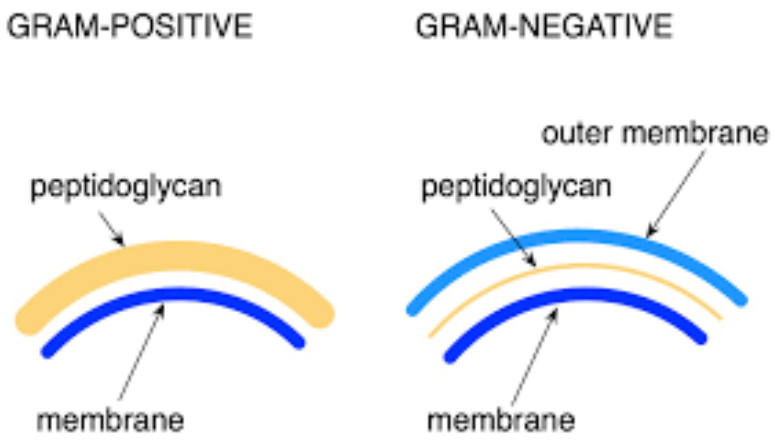
Purpose

The Gram staining method, named after the Danish bacteriologist who originally devised it in 1882 (published 1884), Hans Christian Gram, is one of the most important staining techniques in microbiology . The Gram stain, used to distinguish between Gram-positive and Gram-negative cells, is the most important and widely used microbiological differential stain. In addition to Gram reaction, this stain also allows determination of cell morphology, size, and arrangement. It is typically the first differential test run on a specimen brought into the laboratory for identification. In some cases, a rapid, presumptive identification of the organism or elimination of a particular organism is possible .

Principle

The Gram stain is a differential stain in which a decolorization step occurs between the application of two basic stains. The Gram stain has many variations, but they all work in basically the same way. The primary stain is crystal violet. Iodine is added as a mordant to enhance crystal violet staining by forming a crystal violet–iodine complex. Decolorization follows and is the most critical step in the procedure . Gram- negative cells are decolorized by the solution (generally alcohol or acetone) whereas Gram-positive cells are not . Gram- negative cells can thus be colorized by the counterstain safranin. Upon successful completion of a Gram stain, Gram positive cells appear purple and Gram- negative cells appear reddish pink .

The ability to resist decolorization or not is based on the different wall constructions of Gram-positive and Gram- negative cells. Gram-negative cell walls have a higher lipid content (because of the outer membrane) and a thinner peptidoglycan layer than Gram positive cell walls. The alcohol/acetone in the decolorizer extracts the lipid, making the Gram-negative wall more porous and in capable of retaining the crystal violet– iodine complex, thereby decolorizing it. The thicker peptidoglycan trap the crystal violet–iodine complex more effectively, making the Gram-positive wall less susceptible to decolorization .



Procedure:

1. A heat-fixed bacterial smear is first covered completely with a few drops of a solution of crystal violet, a purple basic dye.
2. After 30-60 sec the smear is rinsed with water by squirting the slide above the smear and letting the water wash over it until the water runs clear.
3. Several drops of iodine (the mordant) are applied to cover the smear and left for 60 sec., then rinsed again.
4. A few drops of an alcohol-acetone mixture or similar solvent are added at a time until the wash is colorless, then the slide is rinsed again.

5. A red basic dye, aqueous safranin, is applied for 30-60 sec. followed by a rinse.
6. The smear is blotted (not wiped) to remove excess water, using bibulous (absorbent) paper or a paper towel. The slide is then air dried the rest of the way.
7. Examine under the oil immersion lens.