Gram Staining

Objective

To perform the gram staining for the given sample by Gram Staining Procedure

Principle of Gram Staining

The differences in Gram-positive and Gram-negative bacteria cell wall composition account for the Gram staining differences. Gram-positive cell wall contains a thick layer of peptidoglycan with numerous teichoic acid cross-linking, which resists decolourization. In aqueous solutions, crystal violet dissociates into CV+ and Cl – ions that penetrate through Gram-positive and Gram-negative cell walls. The CV+ interacts with negatively charged components of bacterial cells, staining the cells purple. When added, iodine (I- or I3-) interacts with CV+ to form large crystal violet-iodine (CV-I) complexes within the cytoplasm and outer layers of the cell. The decolorizing agent (ethanol or an ethanol and acetone solution) interacts with the lipids of both gram-positive and gram-negative bacteria membranes.

The outer membrane of the Gram-negative cell is lost from the cell, leaving the thin peptidoglycan layer exposed. With ethanol treatment, gram-negative cell walls become leaky and allow the large CV-I complexes to be washed from the cell. The highly cross-linked and multi-layered peptidoglycan of the gram-positive cell is dehydrated by the addition of ethanol. Thus ethanol treatment traps the large CV-I complexes within the cell. After decolorization, the gram-positive cell remains purple. In contrast, the gram-negative cell loses the purple color and is only revealed when the counterstain, the positively charged dye safranin, is added.

Preparation of the smear

1. Take a clean grease free slide.
2. Transfer a loop of the liquid sample for curd or sputum or CSF or pus etc to the microscope slide. If performing a Gram stain from a bacterial colony, first put a drop or a few loopful of water and emulsify the bacterial colony in the water drop.
3. Spread the sample to an even-thin film over a circle of 15 mm diameter.
4. Air dry the sample, and once the sample gets air dried, heat fix the smear by passing it through a bunsen burner three times. Heat application helps the cell adhesion (fixation) to the glass slide and prevents its loss during rinsing.

Gram Staining Procedure

The gram staining procedure involves four major steps; staining with crystal violet, fixing the dye, applying a decolourizer and counter-staining.

1. Flood air-dried, heat-fixed smear of cells for 1 minute with crystal violet staining reagent. Please note that the quality of the smear (too heavy or too light cell concentration) will affect the Gram Stain results.
2. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
3. Flood slide with the mordant Gram's iodine and allow to react for 1 minute.
4. Wash slide in a gentle and indirect stream of tap water for 2 seconds.
5. Flood slide with decolorizing agent acetone-alcohol decolorizer and add drop by drop to slide until the decolorizing agent running from the slide is clear.
6. Flood slide with a counterstain, safranin and allow to react for 30 to 1 minute.
7. Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent.
8. Allow the slide to air dry by tilting it onto a paper towel or over a sink. Alternatively, gently dry the slide by blotting it using a lint-free bibulous paper. Please do not use a wiping motion, as it can remove the smear.
9. Focus the slide under the microscope using 10x followed 40x and 60x.
10. Then, without removing the slide, switch to the high-power oil immersion objective lens marked 100x.Gram staining This will result in an overall magnification of 1,000x.

Results

- Gram-negative bacteria will stain pink/red
- Gram-positive bacteria will stain blue/purple