GRAM STAIN

Principle

Bacteria can be recognized as gram positive (blue-black/purple) if they retain the primary dye complex of crystal violet and iodine in the face of attempted decolourization, or as gram negative (pink) if decolourization occurs as shown by the cell accepting the counterstain safranin.

Generally the mechanism of the Gram stain is: The fixed bacteria are stained with the triphenylmethane dye, crystal violet. Next the smear is flooded with Grams solution which oxidatively forms an insoluble complex with the crystal violet. The smear is then flooded with the organic solvent, acetone-alcohol. Depending on cell permeability the crystal violet-iodine complex will be washed from Gram negative bacteria in solvent but not from Gram positive bacteria. Upon counterstaining with safranin, organisms which had been discolorized by the ethanol (Gram negative) will stain pink. Gram positive organisms which retained the crystal violet will appear blue-black/purple microscopically.

Materials

Crystal violet solution
Grams Iodine solution
Acetone alcohol
Safranin solution

Procedure

1. Prepare the film on the slide and allow to air dry.
   DO NOT HEAT TO DRY FILM.

2. When film is dry, place slide on heating block for several minutes. Slide should be just warm to your hand.
   DO NOT OVERHEAT.

3. Allow slide to cool - this will happen quickly - in just a few seconds.
   DO NOT ADD STAIN TO HOT SLIDE.

4. Flood slide with crystal violet - leave 1 minute.

5. Wash gently with water.

6. Flood slide with Grams Iodine - leave 1 minute.

7. Wash iodine from slide with acetone-alcohol mixture. Add a few more drops of acetone-alcohol until no more colour comes from film - usually 30 seconds.

8. Wash gently with water.

9. Flood slide with safranin - leave 1 minute.

10. Wash gently with water. Clean back of slide with tissue and place slide in tray.

Precaution

1. At no time should the film (smear) be exposed to too much heat. When the specimen is still wet, heat causes coagulation of the protein resulting in heavy overstaining which cannot be removed by the decolourizer. A thick smear will also show more tendency to "lift off" during staining.

2. Rinsing the Grams Iodine off with the decolorizer gives more stability to the CV-GI complex and false over decolorizing will not take place.

3. Flooding a hot slide with crystal violet will cause the stain to precipitate and make decolourizing much more difficult.

Quality Control

It is recommended that controls be run concurrently with unknowns or at least run on a daily basis using known smears containing Gram positive and Gram negative bacteria.