



Gram stain Protocol

Materials:

- Hairdryer to heat fix sample
- Crystal violet stain
- Iodine solution
- Decolouriser (ethanol/acetone)
- Safranin counterstain
- Slide rack (or clothes pegs)

Other: As per diff quick materials, if you don't have a slide rack, consider using clothes pegs on both ends of the slide to keep it stable during staining.

Protocol

1. Heat fix the sample with a hair dryer.
2. Place slide on a rack over the sink to minimise spillage of reagents (or use pegs as above).
3. Flood slide with Crystal Violet stain, leave for 1 minute, then rinse with water.
4. Shake off surplus water and apply Iodine solution, leave for 1 minute, then rinse with water..
5. Tilt slide and decolourise. 1-2 drips at a time until no more colour comes off. Do this slowly over 1-2 minutes to allow adequate penetration of the smear.
6. Apply Safranin counterstain, leave for 2 minutes, then rinse.
7. Blot or air dry and examine under oil immersion.

Crystal Violet
Flood slide, 1 minute, then rinse and shake off excess water.

Iodine solution (mordant)
Flood slide, 1 minute, then rinse.

Alcohol wash (decolorization)
Tilt slide, drip decolorise until no more colour comes off (1-2 minutes).

Safranin O (counterstain)
Flood slide, 2 minutes, then rinse.

Gram positive = Purple

Gram negative = Red