

Gram stain Protocol

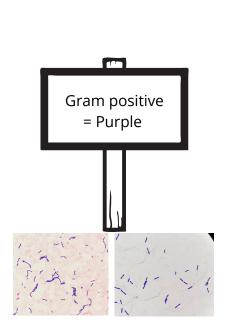
Materials:

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.

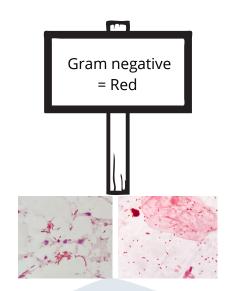
Protocol

- 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 lodine 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.





Safranin O Flood slide, 2 minutes, then rinse.



For a video of this procedure please visit www.amrvetcollective.com/home/continuing-education.