Gram staining technique

1) For smears from solid medium (e.g. nutrient agar plates or slopes, emulsify a very small portion of a single colony in a drop of 0.9% saline on a clean plain microscope slide. For smears from liquid media (e.g. nutrient broth), transfer a loopful of fluid to a clean microscope slide. This will contain far fewer organisms than smears of colonies from solid media.

2) Allow material on slide to completely air dry.

3) Pass the slide quickly through a Bunsen flame (approximately 0.5 seconds) to fix the material to the slide. Do not overheat.

4) Place a large drop of crystal violet on the slide, over the smear. Leave the stain on the slide for 1 minute.

5) Rinse the slide under running tap water for 5 seconds.

   NOTE: At each rinsing, take care not to wash smear off the slide.

6) Cover the smear with Gram's iodine solution and leave for one minute.

7) Rinse the slide under running water for 5 seconds.

8) Decolorise briefly with acetone alcohol for 2 seconds.

9) Rinse under running water for 5 seconds.

10) Counter stain by completely covering the smear with Safranin stain for 30 seconds.

11) Rinse briefly with running water and blot slide dry. A piece of white clean paper can be used to blot the slide.

12) Gram positive species will appear blue to purple, while Gram negative species will appear red to pink.

    Disposal: At the end of a microbiology activity it is important that all equipment be sterilised prior to disposal. Sterilise at 121^{o}C and a pressure of 15 psi for 20-30 minutes prior to disposal. Verify conditions with a sterile confirmation strip (E5.36).

    For safe laboratory handling and disposal, refer to AS/NZS2243 Safety in Laboratory Standards.