Note: Avoid picking up too much growth as this will result in a heavy smear, which will not stain effectively and cannot be examined with accuracy.

Note: You may wish to vortex a broth culture prior to creating a smear as this will ensure an even mix of cells in the culture. As the broth is a liquid culture, water is not required to create the smear.

3.1.2. Allow the smear to air dry and heat fix the cells to the slide by passing the slide through the hottest part of the flame three times. Allow to cool.

3.1.3. Flood the slide with Crystal Violet solution and stand for 30 seconds (Fig. 2A).

3.1.4. Rinse off Crystal Violet solution with water and tilt the slide to drain excess water.

3.1.5. Flood the slide with 1% Lugol's Iodine and stand for 30 seconds (Fig. 2B).

3.1.6. Rinse off Iodine with water and tilt the slide to drain excess water.

3.1.7. Flood the slide with Decolourising solution and immediately rinse the slide with running water after 3 seconds (Fig. 2C). Tilt the slide to drain excess water.

Note: Prolonged contact with decolourising solution will result in over-decolourisation of cells and a false gram reaction. Ensure the contact time is limited to ***3 seconds only****.*

3.1.8. Flood the slide with the desired counterstaining solution. Leave to act for *up to* 2 minutes (Fig. 2D).

3.1.9. Rinse with water and blot dry using a paper towel. Do not rub.

3.1.10. Examine the smears under Oil Immersion.

3.1.11. Record the cellular arrangement (see Appendix 7.2) and Gram stain result of the organism on the Isolate Identification Record form.

3.1.12. Interpretation

- **Gram-positive** Gram-positive organisms stain purple
- **Gram-negative** Gram-negative organisms stain pink/red.

3.1.13. Quality Control

3.1.13.1. Gram-positive and Gram-negative reference cultures are to be used for quality control of the gram staining procedure and reagents.

- **Gram Positive** *Staphylococcus aureus ATCC 6538*
- **Gram Negative** *Escherichia coli ATCC 8739*

3.2. **Technical Information**

Some Gram-positive bacteria may appear Gram-negative in whole or in part eg. some strains of Gram-variable *Bacillus and Clostridium*. Yeasts will appear "Gram-positive" and will be visibly much larger than a prokaryotic bacterial cell.

All Gram-variable and suspected over or under-decolourised organisms are to be subject to a KOH test (see section 3.3).