

Y12 Diploma Task

Learning Intention:

- To identify gram negative and gram positive bacteria through staining.

Activities

- Discuss the role of Gram staining when diagnosing infection.
- Prepare a streak plate to cultivate isolated colonies of bacteria from a stool sample.
- Prepare a mixed smear of Escherichia Coli (Gram-negative) and Bacillus subtilis (Gram-positive) and carry out a Gram stain procedure.
- Discuss the results from a Gram stain on a stool sample from a patient suffering from diarrhoea.

Method

1. Label a microscope slide using a permanent marker pen.
2. Use an inoculation loop to transfer sterile water onto the labelled side of the slide, repeating the process to obtain a drop of water 1 cm in diameter.
3. Using an inoculation loop, take a small sample of the first culture and mix it with the water on the slide.
4. Then take a sample of the second culture and spread it across the slide. Ensure it is mixed with the first culture.
5. Dry the smear by holding it in the warm air about a meter above a Bunsen flame.
6. Fix the bacteria by passing the slide very rapidly through the hottest part of the roaring Bunsen flame (transit time less than 1 second). Repeat this twice.
7. Cover the slide with crystal violet stain and leave it on for 1 minute
8. Wash off the crystal violet stain with Lugol's iodine.
9. After washing off the crystal violet, leave the iodine solution to stand on the slide for 1 minute.
10. Use the ethanol to wash off most of the iodine into the tray.
11. Move the slide to the white tile, then continue rinsing until the washings are a very pale violet colour.
12. Wash off the ethanol with distilled water from the wash bottle.
13. Apply the Safranin counter-stain and leave on for thirty seconds.
14. Wash off the safranin with distilled water from the wash bottle.
15. Blot the slide very gently using filter paper until it is dry.
16. View under a microscope.

Summer Task – Part 1

- Annotate a detailed diagram illustrating how does the structure of bacteria affect gram staining?

Summer Task – Part 2

- Research the effect of antibiotics on gram negative and gram positive bacteria?
- Research the effect of antibiotic resistance on infectious diseases and hospital transmitted infections.