

Mi3 : Smear preparation and Gram stain

Requirement for one experiment

5 mL of mixture of (E.coli + Staphylococcus aureus) broth

One set for Gram stain :

- * one 100 mL-flask of gentiane violet
- * one 100mL-flask with lugol
- * one 100mL-flask with ethanol
- * one wash bottle
- * one 100mL-flask with safranine
- * one microscope and immersion oil

Generalities

Bacteria can be classified according to many parameters :

- their shape : cocci / rods
- their metabolism : heterotrophic / autotrophic...
- the structure of their cell wall according to their Gram stain reaction : Gram positive and negative : this system is adopted above

Bacteria are prokaryotic micro-organisms, i.e. the genome, DNA, is not contained in a nucleus and there is no nuclear membrane.

Bacteria cell wall can be either thin or thick ; cell wall structure surrounds cytoplasmic membrane

1. Smear preparation : page 1

After fixing the smear, cool it.

2. Gram stain : page 2

Modifications :

- 2 : don't rinse, just discharge the excess of crystal violet
- 3 : cover with KI during the same time than crystal
- 6 : rinse IMMEDIATELY
- 9 : the glass slide should be completely dry

Add a little immersion oil ; observe with the objective X100.

Report :

Observation of the gram stain :

- is the culture pure ?
- Gram positive or negative ?
- shape : cocci, rod ?
- association : chain, cubes...
- average size (1 to 10µm) ?