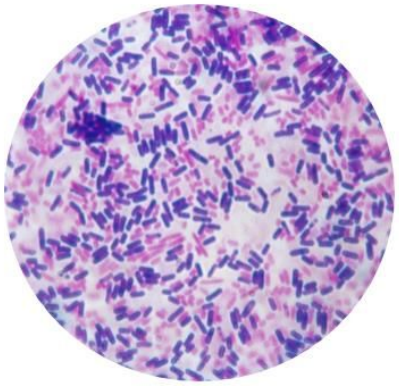
Gram Stain

Results & Observations



Organism: P.aeruginosa, S.aureus, mixture

Purpose

- Differential stain that is used to distinguish between Gram-negative (pink or red) and Gram-positive cells (purple or blue).
- Allows determination of cell morphology, size and arrangement.
- Choice of antibiotics can be made on the basis of a Gram stain report.

Procedure

1. Primary stain - Crystal Violet -(1-2 min, then rinse)
2. Mordant - Gram's Iodine - (1-2 min, then rinse)
3. Decolorizer -95% Alcohol solution - 10-30 seconds (rinse)
4. Counterstain -Safranin (1-2 min, then rinse)
5. Blot slide dry gently with bibulous paper

Interpretation & Questions:

1. **What would you observe if you decolorized your slide too much? How would your cells appear?**
   All of your cells would appear pink if you decolorized your cells too much with acetone-alcohol.
   Acetone-alcohol, if used too liberally, will wash the crystal violet out of the cells in your smear. There would be little distinction between gram-positive and gram-negative cells.
2. **How would you describe the morphology and arrangement of the cells in your stained preparations?**

- *Staphylococcus aureus:* gram-positive (purple) cocci that form grape-like or staphylo- arrangements of cells.
- Pseudomonas aeruginosa - gram-negative (pink) bacilli.

3. **You are looking at the smear of the mixture. All the cells, cocci and bacilli, appear deep purple. What could have gone wrong?**
   You probably did not properly decolorize or forgot to decolorize with acetone-alcohol. Using water instead of acetone-alcohol in the decolorization step would produce this result.
4. **Explain the relationship between the observed Gram reaction and bacterial cell wall structure.**
   In a gram positive cell there is more peptidoglycan then in a gram negative cell. The gram reaction dyes more of the gram positive because there is more peptidoglycan. Then going through the process gram negative gets no color until safranin.
5. **You mistakenly confuse the primary stain and counterstain. You initially stain the smear with safranin, add iodine, and then decolorize and counterstain with crystal violet. How does your**

mixed culture now appear when viewed with oil immersion?
They all would look purple and it would be very hard to distinguish between the two.