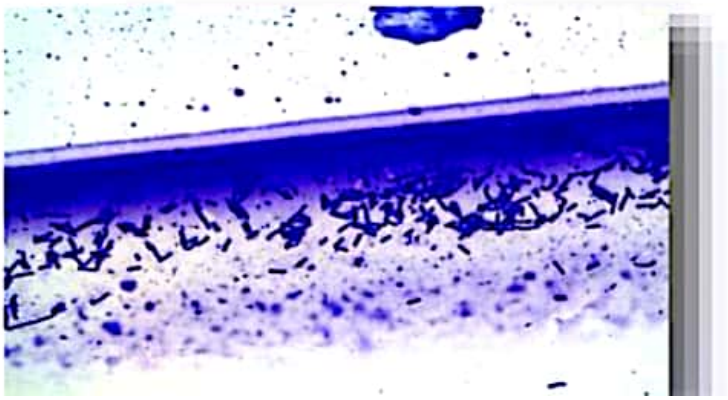
Gram staining

Principle:

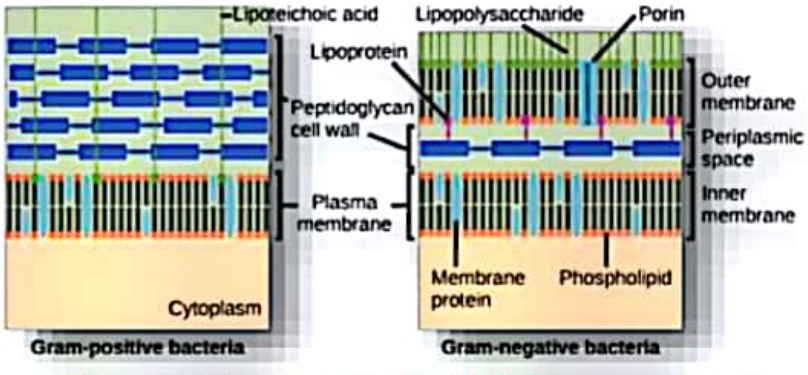
- Gram staining is the most important differential staining used in bacteria, named after Dr. Christian Gram.
- It differentiates bacteria into two groups- Gram positive and Gram negative.
- Differential staining requires three reagents that are applied in sequence to the heat fixed smear.
- The first reagent is the primary stain. Its function is to impart colour to the cell. Here the crystal violet stain is used which imparts the violet colour to the cells.



- In order to fix the colour, a mordant (Gram's iodine) is used. It forms an insoluble complete binding to the primary stain.
- The resultant crystal violet-iodine complex serves to intensify the colour of the cell. All the cells appear purple black.

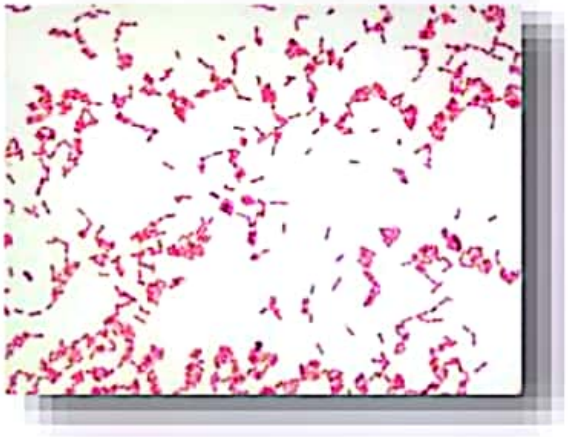
- In Gram positive organisms, the crystal violet-iodine(CV-I) complex binds to Mg-RNA to form MgRNA-CV-I complex.
- It is more difficult to remove than mere CV-I complex.
- 90% alcohol serves as decolourising agent, lipid solubilizer and as protein dehydrating agent. Its action is determined by Gram positive cells.

- There is low lipid concentration in Gram positive bacteria, since low quantity of lipid content is readily discovered by alcoholic action causing formation of minute cell wall pores.
- These spores are then closed by alcohol dehydrating effect.
- Therefore it becomes difficult in removing MgRNA-CV-I complex and the cell remain purple.

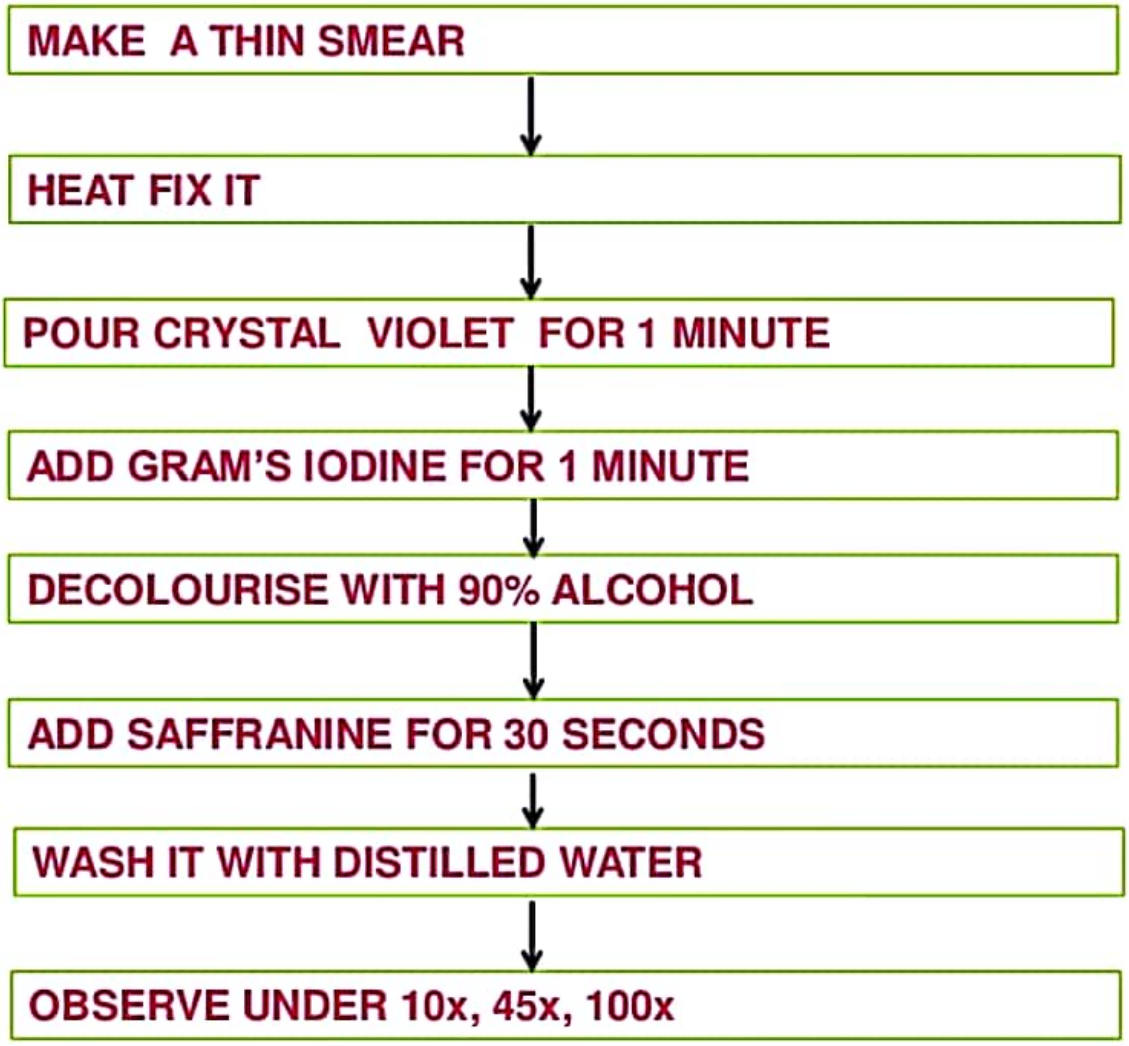


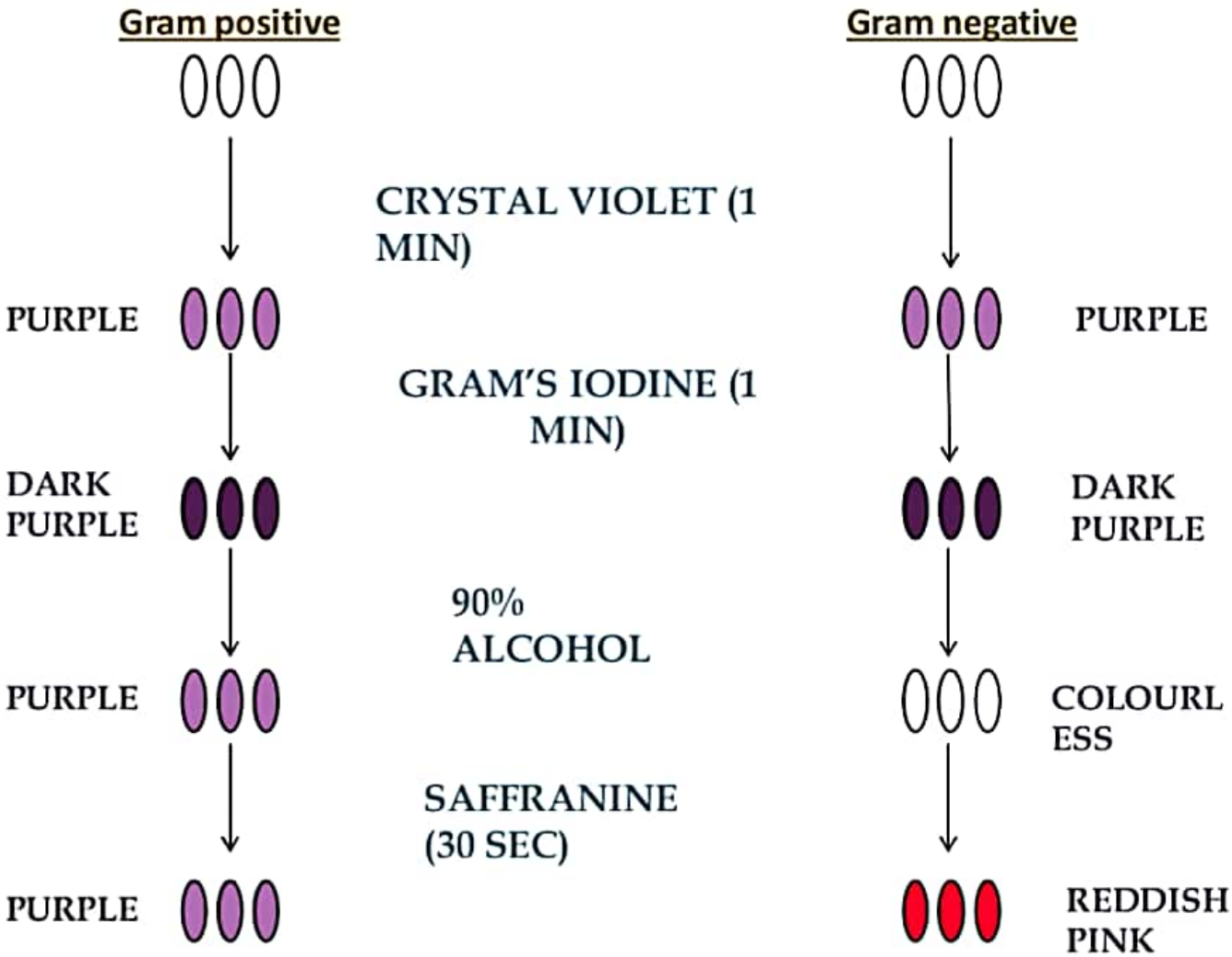
- In Gram negative cells, the high lipid concentration found in the outer membrane of the cells is dissolved by alcohol, creating large pores on the cell wall that does not close appreciably on dehydration of cell wall proteins.
- This facilitates release of unbound CV-I complex leaving the cell colourless or unstained.

- **The final reagent is the counter stain Saffranine. It stains the cell pink which were previously colourless.**



PROCEDURE





GRAM POSITIVE