Safety rules & Gram stain method

Disinfectants

The bench that you are working on must be wiped with a disinfectant or sterilizer, such as Dettol or 70% alcohol

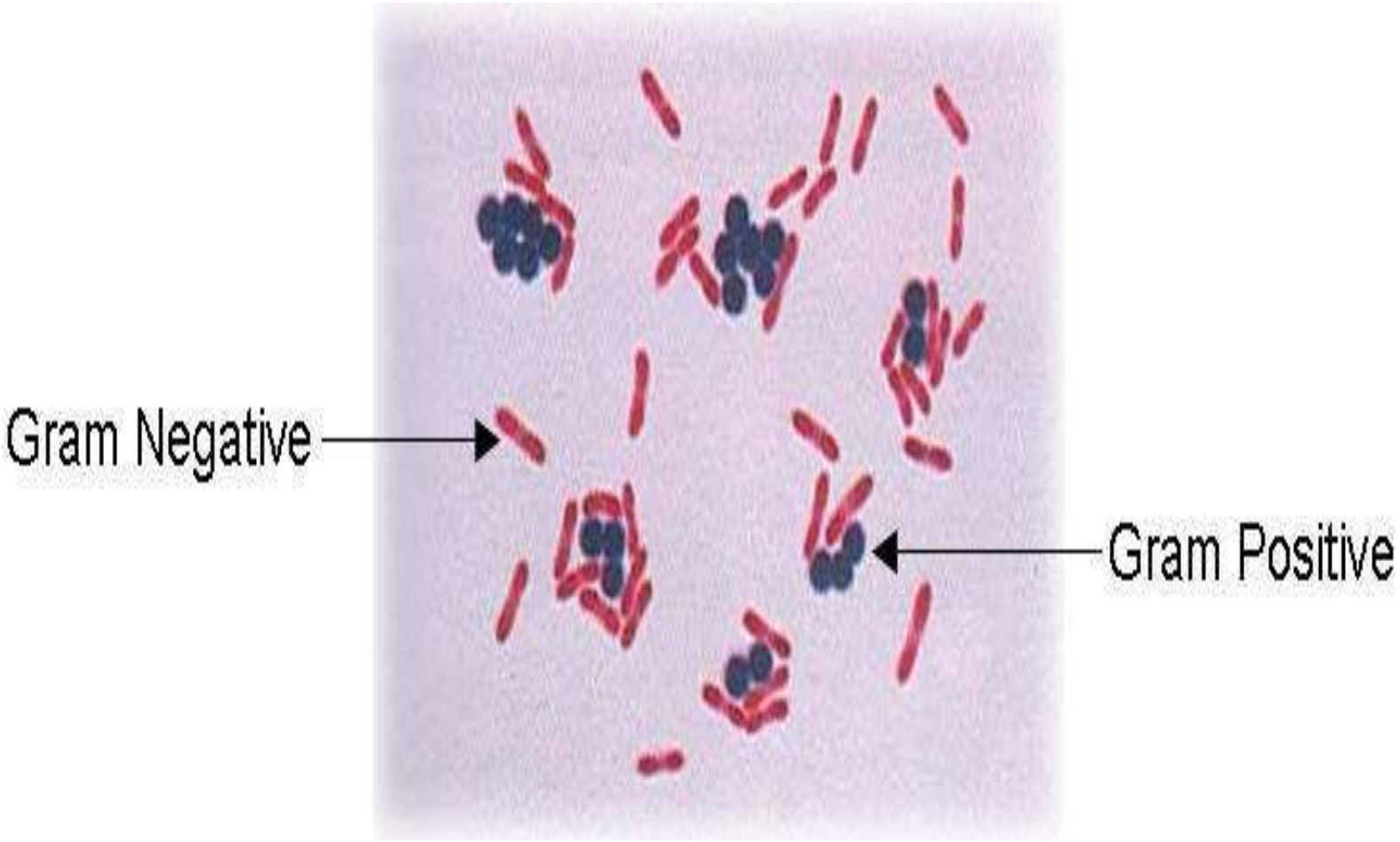
we use 70% alcohol not 100% alcohol because it was found that 70% alcohol kills most types of bacteria. Bacteria could survive in 100% alcohol

You must wear a lab coat in order to prevent transmission of infections and to protect yourself against spills of liquids or cultures

Food and drinks are not allowed in the lab

The Bunsen burner is important especially in heating because this can help in making experiments, in boiling water in test tube. It sterilizes the workplace and the slides used and the air surrounding the workplace

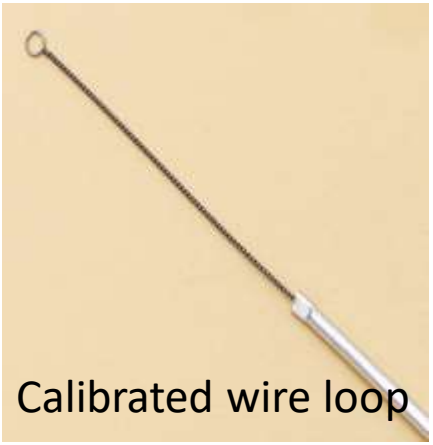
Gram stain

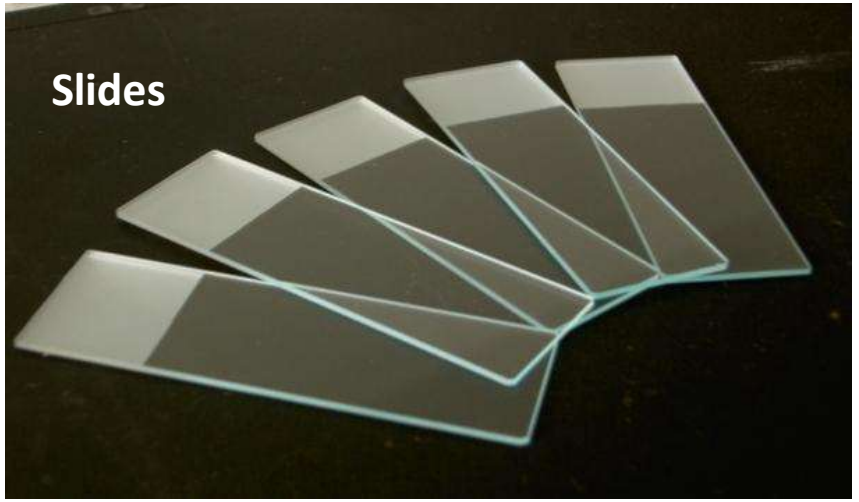


the Gram stain is the basis of microbiology. It's effective for classification of bacteria. this classification helps in the diagnosis and treatment of bacterial diseases.
The bacteria is divided into two types: Gram Positive and Gram Negative

Gram stain tools

Four reagents are used in Gram stain: Crystal violet, Iodine, Alcohol (95%), Safranin.





Media for the bacteria to grow

The calibrated wire loop:
Calibrated means that it has a certain volume in microliter (µL). Before using it, it must be heated on the flame; when it turns red we know that all that is on it got burnt and that it is sterile and ready for use.

There are 2 types of media that can be used to isolate the bacteria and let it increase in number.
1. Semi-solid agar (petri dishes). The bacteria growth in Colonies.
2. Liquid media (broth) placed in glass tubes. The bacteria growth in Turbidity.

In order to gram stain a culture, you can either take a sample from broth media (liquid) using a wire loop, and place the drop on a slide and spread it, or take a sample from the colonies on agar (semi-solid) where it is spread and diluted on the slide.
After that, we have to leave the slide to air dry for one minute We pass each sample quickly on a burner for fixation. (because the slides will be exposed to a lot of dyes)

A Smear is Prepared by Spreading Bacteria on a Glass Slide

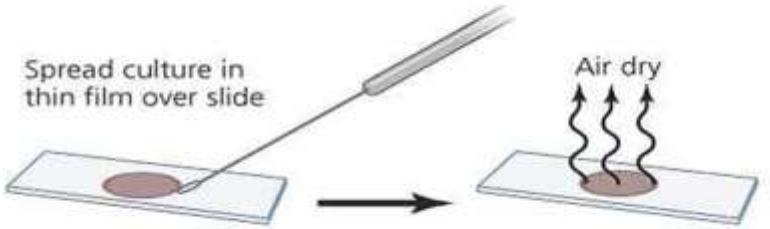
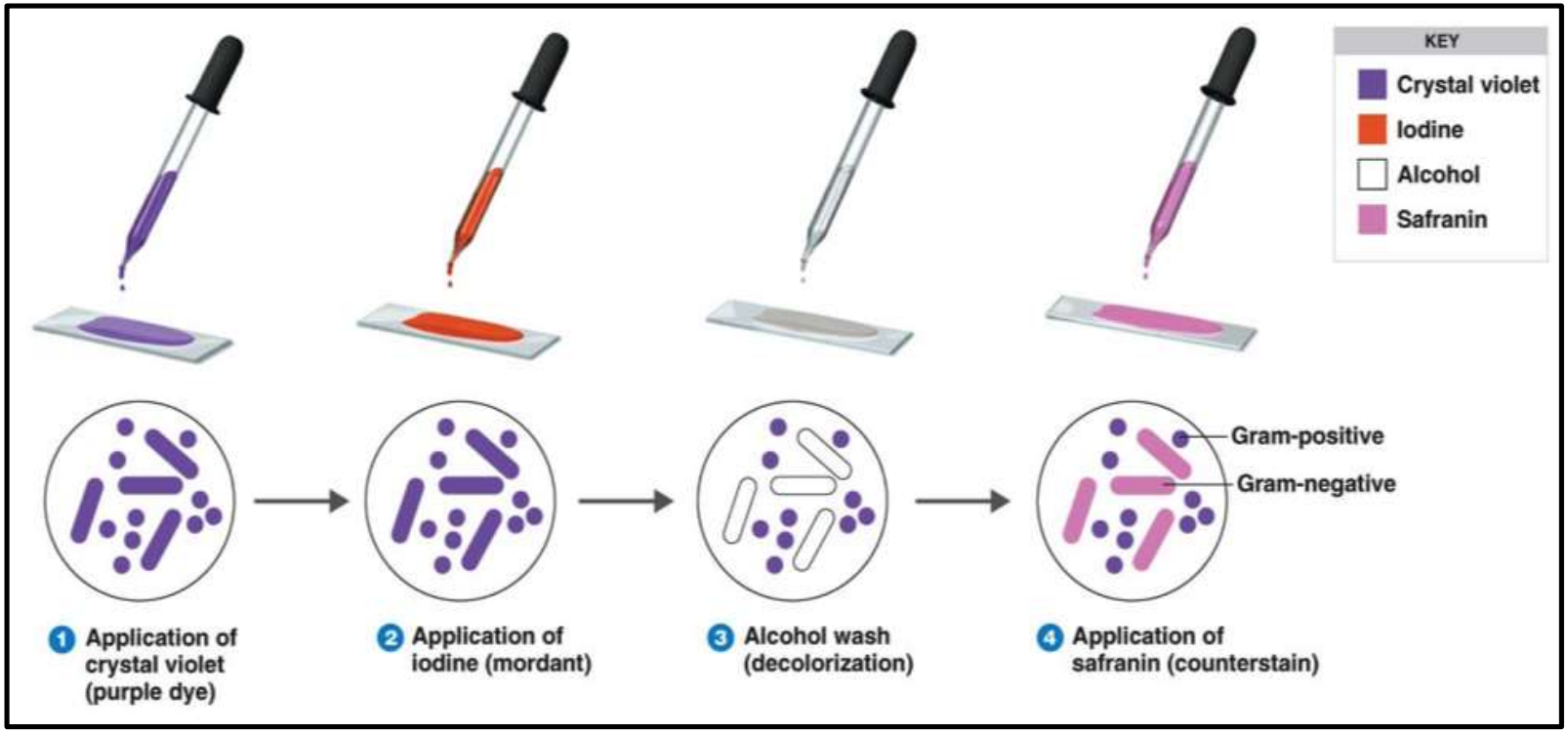


Figure 4.15



Steps For Gram Staining

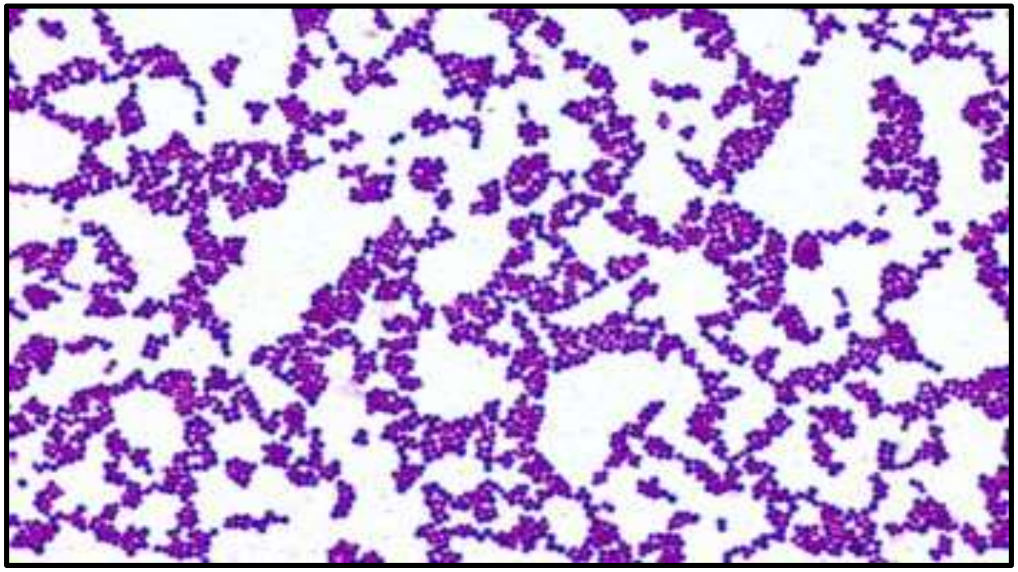
1. **Use the Crystal Violet stain (Purple or Blue) which is a primary stain and keep it for about 30 seconds. And we add it drop by drop to make sure that all the area fixated is stained and that the bacteria absorbed the stain. Then wash it with water. After this step all bacteria changes color from colorless to purple.**

2. **Add Iodine. It is a mordant(fixative) stain. It forms a complex with crystal violet (iodine-crystal complex). The color is still purple.**

3. **Put 95% of Alcohol also drop by drop for 20 seconds then washed it quickly with water. This is a critical step because it is the step that differentiates Gram Positive and Gram Negative bacteria. Now the Gram Positive stay Purple but the Gram Negative become colorless. Why we washed the slide quickly? because the Gram Positive bacteria would also lose its color.**

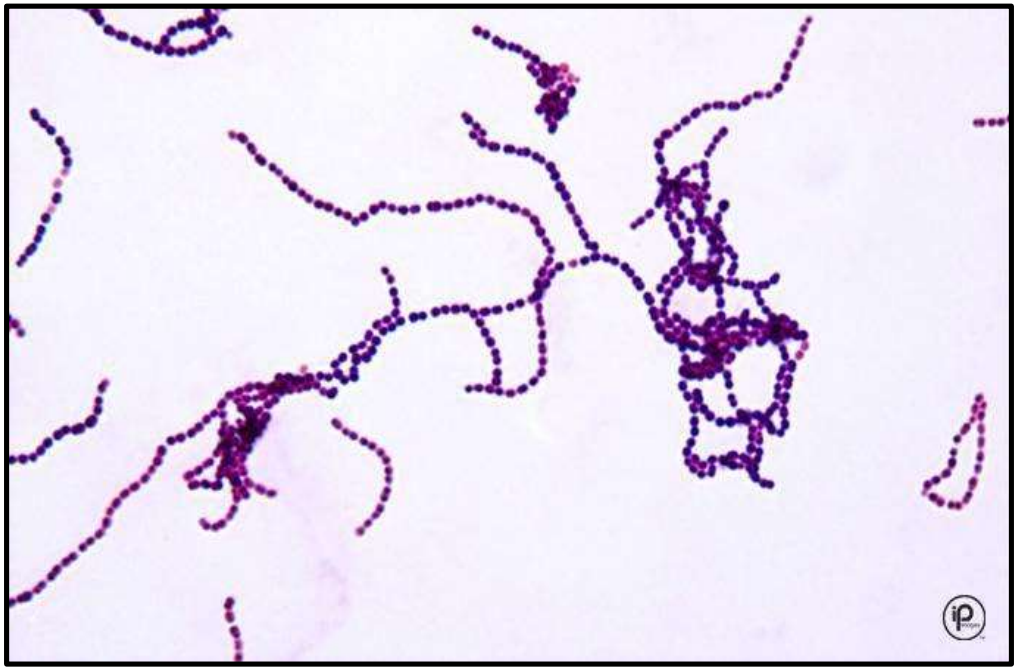
4. **Use a counter stain; any stain that is not violet . Safranin stains Gram Positive bacteria pink. Gram Positive bacteria are already colored so they will stay Purple.**

(Gram Positive)
grape-like clusters
Staphylococcus

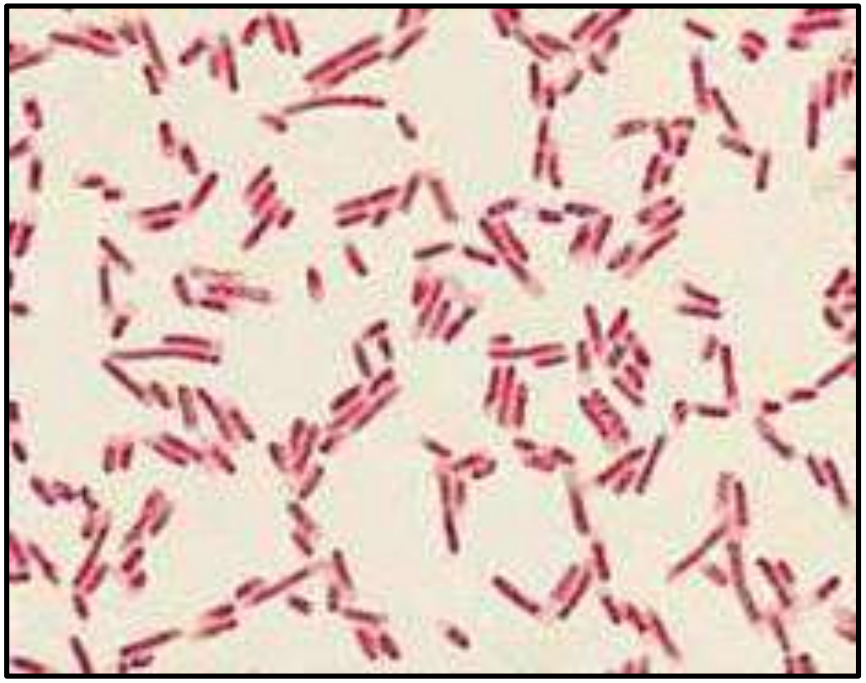


The color and shape of bacteria under the microscope both help in identification.

(Gram Positive)
chains Streptococcus



(Gram Negative)
rod shaped



(Gram Positive)
rod shaped