MICROBIOLOGY LAB #1
SAFETY RULES & GRAM STAIN METHOD

Precaution processes are extremely important when working with cultures in the lab for the safety of the microbiologist from getting diseases from bacteria being worked with. Sterilization and disinfection are also very important in order to attain precise results when dealing with bacteria.

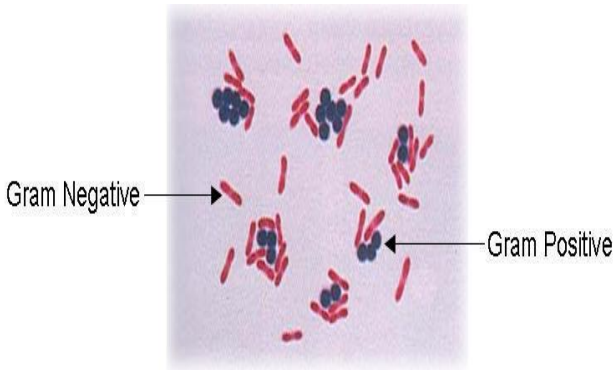
- You must wear a lab coat in order to prevent transmission of infections and to protect yourself in case of spillage of cultures.
- The bench that you are working on must be wiped with a disinfectant or sterilizer, such as Dettol or 70% alcohol.

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.


- Food and drinks are not allowed in the lab.
- A Bunsen burner flame must be present on the bench at all times. It sterilizes the workplace and the slides used and the air surrounding the workpl

GRAM STAIN

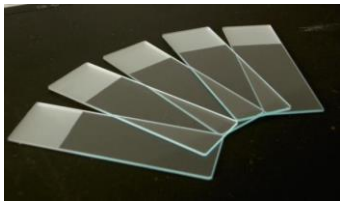
It is the basis of microbiology. It is used to classify bacteria; this classification helps in the diagnosis and treatment of bacterial diseases.
All types of bacteria are either GRAM-POSITIVE or GRAM-NEGATIVE bacteria.



Four reagents are used in Gram stain:

1. Crystal violet.
2. Iodine.
3. Alcohol (96%).
4. Safranin.

We also use reagents, a calibrated wire loop, a Bunsen burner, slides (where we place bacteria in order to view it under the microscope), and media for the bacteria to grow.

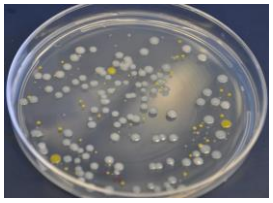


The calibrated wire loop:

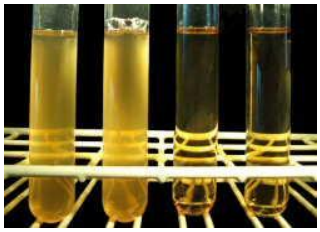
Calibrated means that it has a certain volume in microliter (*μL).* Before using it, it must be heated on the flame; when it turns red we know that all that is on it got burnt and that it is sterile and ready for use.

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). Colonies indicate growth of bacteria.



2. Liquid media (broth) placed in glass tubes. Turbidity indicates growth of bacteria.



The two tubes on the left are turbid, hence they contain bacteria. The two tubes on the right are clear so they do not contain bacteria.

Note: Agar plate bacteria should be transferred to liquid medium by dilution.

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 then use the Bunsen burner flame which fixates the bacteria on the flame.

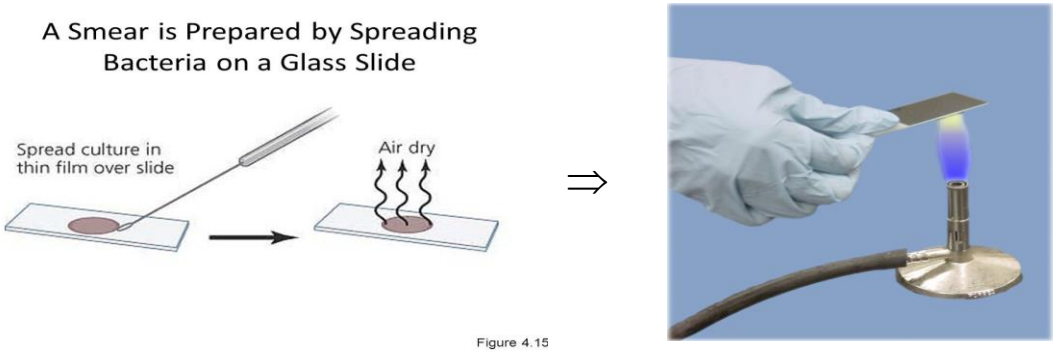


Figure 4.15

*Cells should form thin, barely visible film by smearing the cells obtained from the surface of an agar medium or a fresh culture.
*The bacteria should be fresh because as they age they lose their ability to retain their stain.

QUESTION: Why do we leave it to air dry and not expose it to the Bunsen burner directly?
ANSWER: If we directly expose it on the flame, the bacteria could be shocked resulting in their destruction.

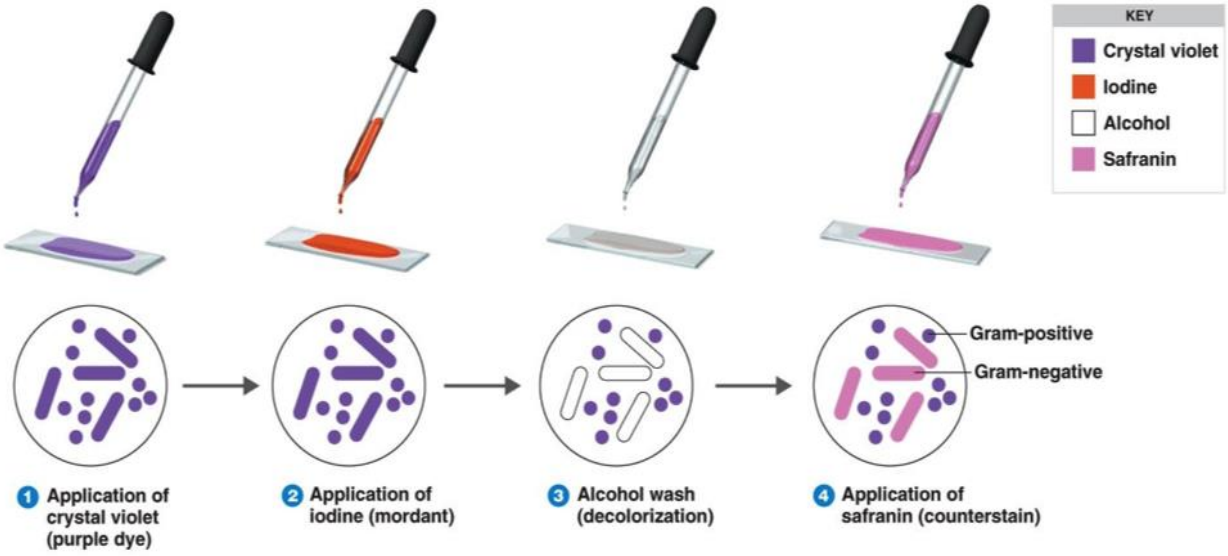
*STEPS FOR GRAM STAINING

1. Use the CRYSTAL VIOLET stain which is a *primary stain*. Its color is dark blue/violet. Add it for one minute, drop by drop, to make sure that all the area fixated is stained and that the bacteria absorbed the stain. Leave it for one minute then wash it with tap water on sink using a holder to remove excess stain. ALL bacteria changes color from colorless to purple.
2. Add IODINE for one minute. It is a *mordant(fixative)* stain; it forms a complex with crystal violet (iodine-crystal complex) decreases solubility in the cell. The color is still blue.
3. This is the critical step in gram staining. It is the step that differentiates GRAM-POSITIVE and GRAM-NEGATIVE bacteria. ALCOHOL (96%) is added drop by drop for 20-30 seconds, then it is directly washed. The complex of GRAM-NEGATIVE disappears, while GRAM-POSITIVE keeps the

complex. The staining depends on the cell wall. GRAM-POSITIVE bacteria have thick layer of peptidoglycan in its cell wall. GRAM-NEGATIVE bacteria have a thin peptidoglycan layer, so the complex can be easily removed. At the moment, on the slide GRAM-POSITIVE bacteria are violet, and GRAM-NEGATIVE bacteria are colorless.

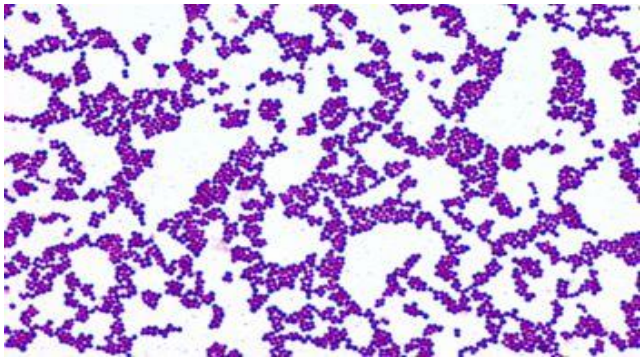
-we can't decolorize for a long time because the GRAM-POSITIVE bacteria would also lose its color.
- the slide should be tilted to an angle to let the droplet move along the slide. Stop doing so when the slide starts to become colorless.

4. Use a *counter stain*; any stain that is not violet could work in order to be able to differentiate. SAFRANIN stains GRAM-NEGATIVE bacteria pink. GRAM-POSITIVE bacteria are already colored so they won't get colored.

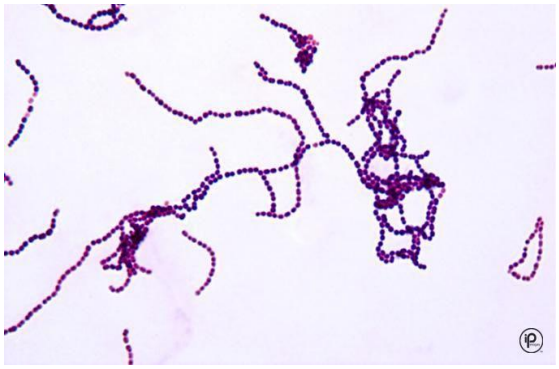


After staining both types of bacteria the slide is washed and dried using filter paper gently. The slide is then viewed under the microscope, where we could determine whether the bacteria cultured is GRAM-POSITIVE or GRAM-NEGATIVE bacteria, if its shape is <u>rod like</u> or <u>cocci</u> or <u>spiral</u>, and the type of aggregation, for

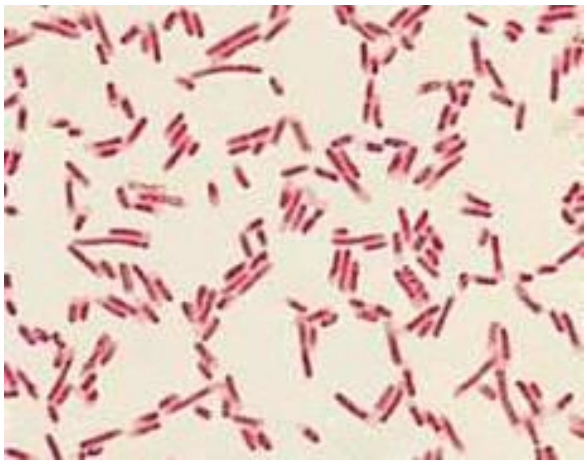
instance, staphylococcus form grape-like clusters and streptococcus form chains.



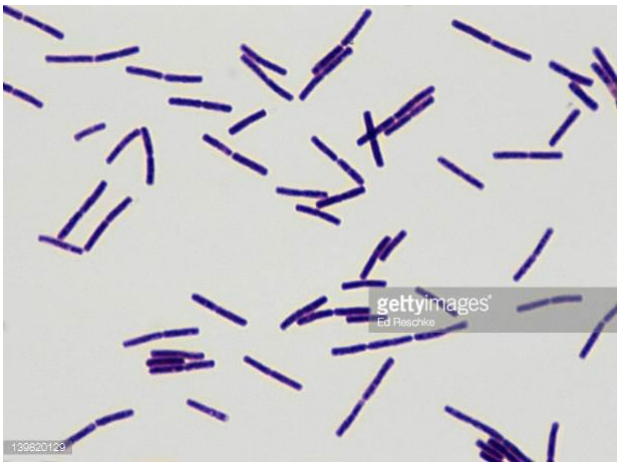
GRAM-POSITIVE grape-like clusters
Staphylococcus



GRAM-POSITIVE chains
Streptococcus



GRAM-NEGATIVE
rod-shaped



GRAM-POSITIVE
rod-shaped

For further understanding watch this video that the doctor recommended.
https://www.youtube.com/watch?v=UbGl3CUboPo&fbclid=IwAR0lM9YXwkdRYNVIOuwrE7pfsXUELT6VGweUtCrq5H80Aa91ObdFoyz2DXo

Best of luck,
Dana Alqatawneh.