

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

140MICRO

LAB 6: SIMPLE STAIN + NEGATIVE STAIN



PURPOSE

- ③ **To recognize the three basic shapes of bacterial cells.**



THE THREE COMMON SHAPES OF BACTERIA:

1-Coccus

2- Bacillus

3- Spiral

1-Coccus

having one of the following arrangements:

- ⊙ Diplococcus: a pair of cocci
- ⊙ Streptococcus: a chain of cocci
- ⊙ Tetrad: a square of 4 cocci
- ⊙ Sarcina: a cube of 8 cocci
- ⊙ Staphylococcus: cocci in

irregular, often grape-like clusters



coccus



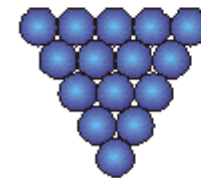
diplococcus



streptococcus



tetrad



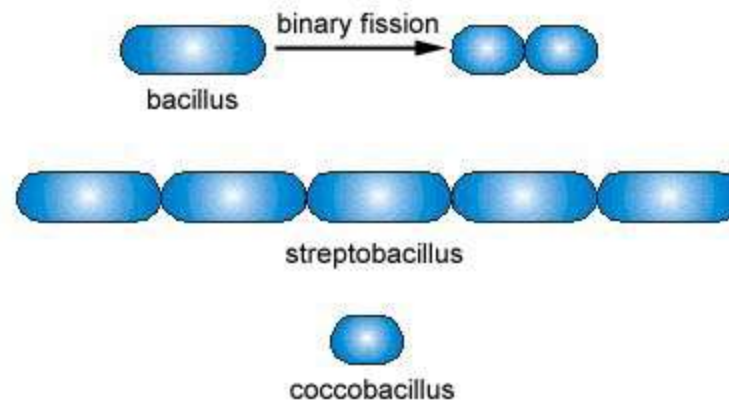
staphylococcus



sarcina

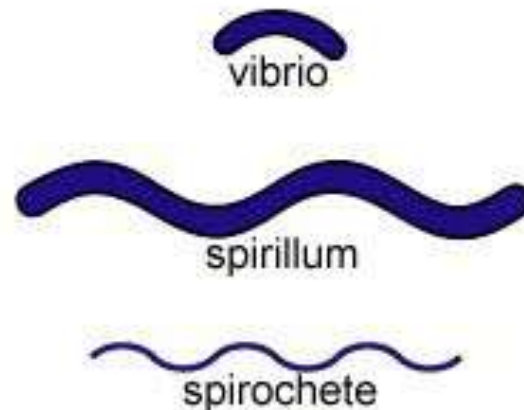
2- Bacillus

- ⊙ Bacillus: a single bacillus
- ⊙ Streptobacillus: bacilli in chains
- ⊙ Coccobacillus: oval and similar to a coccus



3- Spiral

- ③ Vibrio: an incomplete spiral or comma-shaped
- ③ Spirillum: a thick, rigid spiral
- ③ Spirochete: a thin, flexible spiral





SIMPLE STAIN



SIMPLE STAIN :

- ③ The simple stain is a very simple staining procedure involving only one stain.
- ③ You may choose from **methylene blue**, **safranin**, and **crystal violet**.

SIMPLE STAIN :

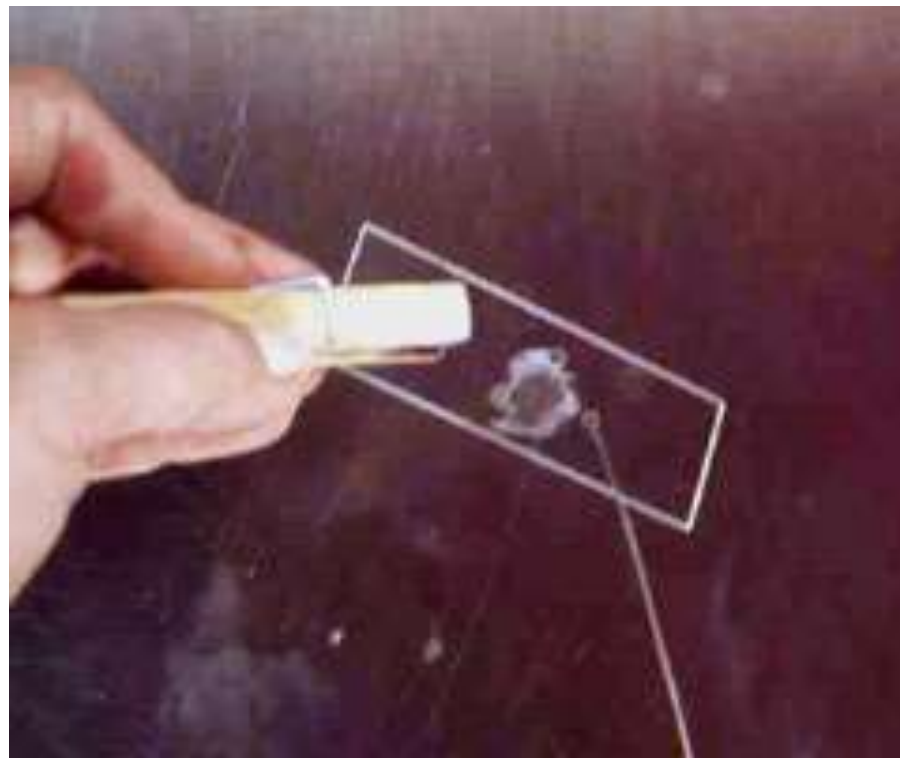


1. Prepare the smear.

- place a small drop of water on a clean slide. Drag the sterile inoculating needle tip through the edge of colony.
- Gently spread the mixture into a circle to spread out.

SIMPLE STAIN :

2. Let the smear air dry completely.



SIMPLE STAIN :



3. Heat-Fix the smear.

- ◎ Smears are heat-fixed by quickly passing the slide through a flame two or three times.
- ◎ **This causes** the microbes to **stick** to the slide and not get washed off during the staining process.

SIMPLE STAIN :



4. **Stain the smear.**

- ③ Place the slide on a rack over the sink. Flood the smear with stain and let it for 60-90 seconds. Rinse gently and blot dry.

SIMPLE STAIN :



5. Then, place a drop of oil directly on the stained smear .Turn the oil lens into position and fine focus to observe the cells.

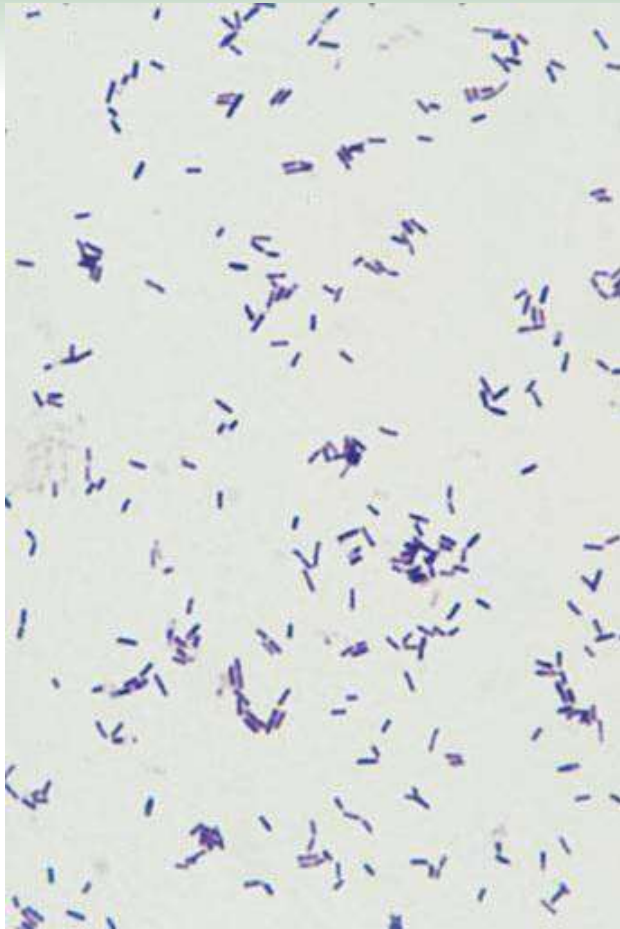
The image features a decorative background with a green top section, a blue left section, and a white bottom section. A light green circle is positioned in the top-left corner. The word "RESULT" is centered in the white section.

RESULT

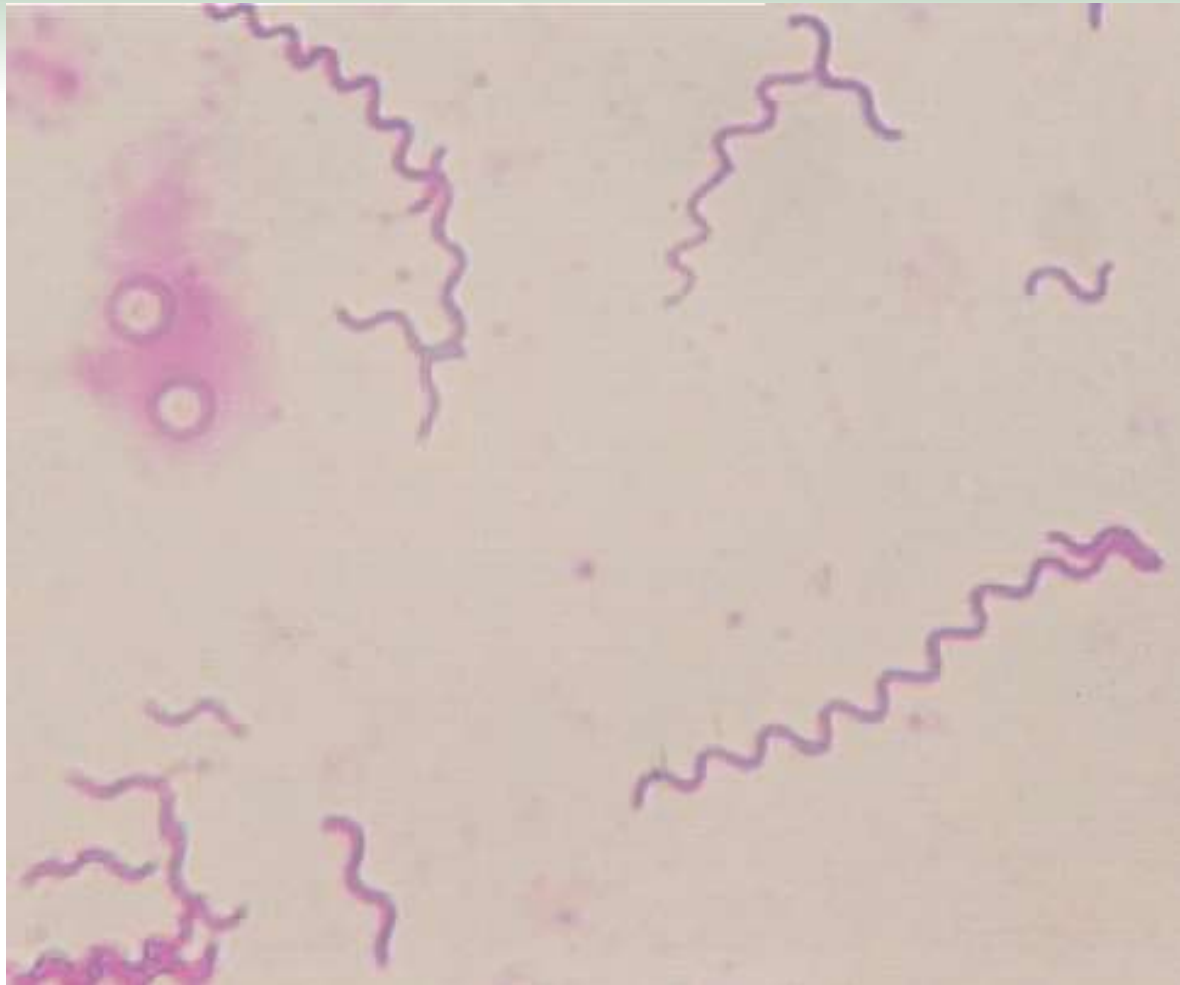
Coccus (cocci pl.)



Bacillus (Bacilli pl.)



Spirillum (Spirilli pl.)





NEGATIVE STAIN

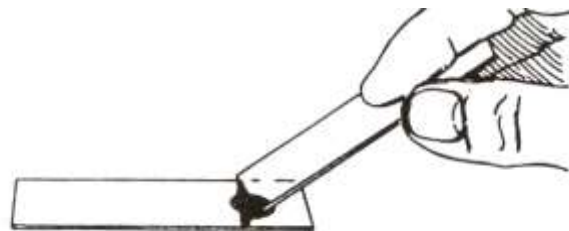
NEGATIVE STAIN :

1. Place a very small drop of **nigrosin** near one end of a well-cleaned and flamed slide.
2. Remove a small amount of the culture with an inoculating loop and disperse it in the drop of stain .

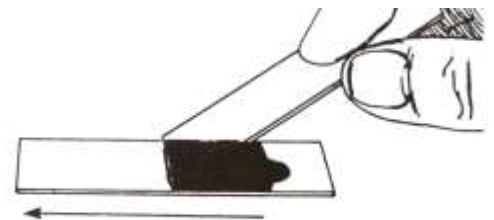


NEGATIVE STAIN :

3. Use another clean slide to spread the drop of stain containing the organism using the following technique.



1



2

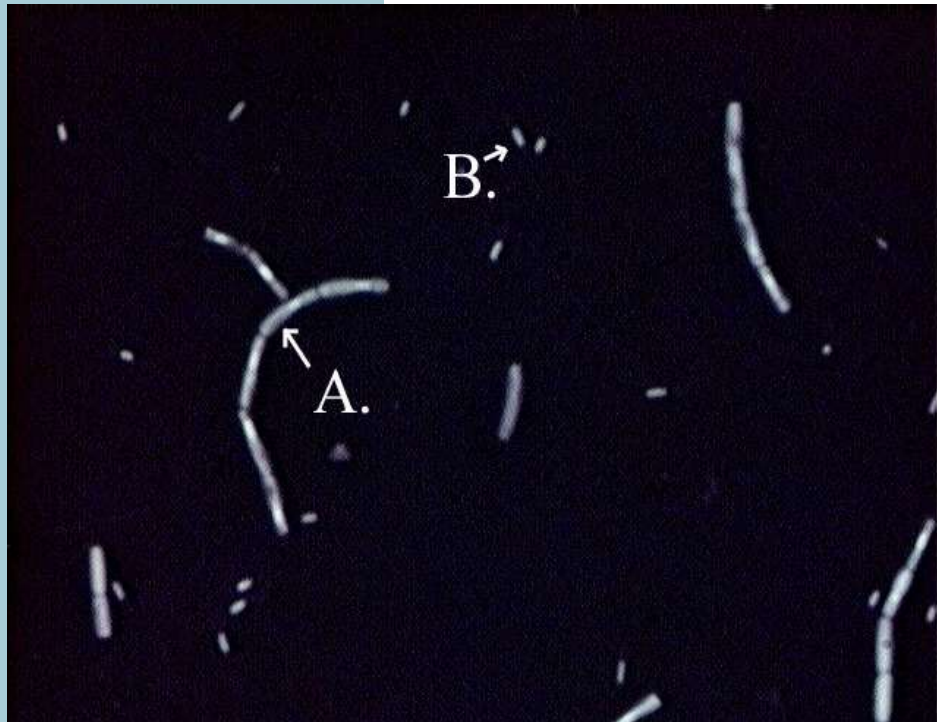
NEGATIVE STAIN :

5. Allow the smear to dry without heating.

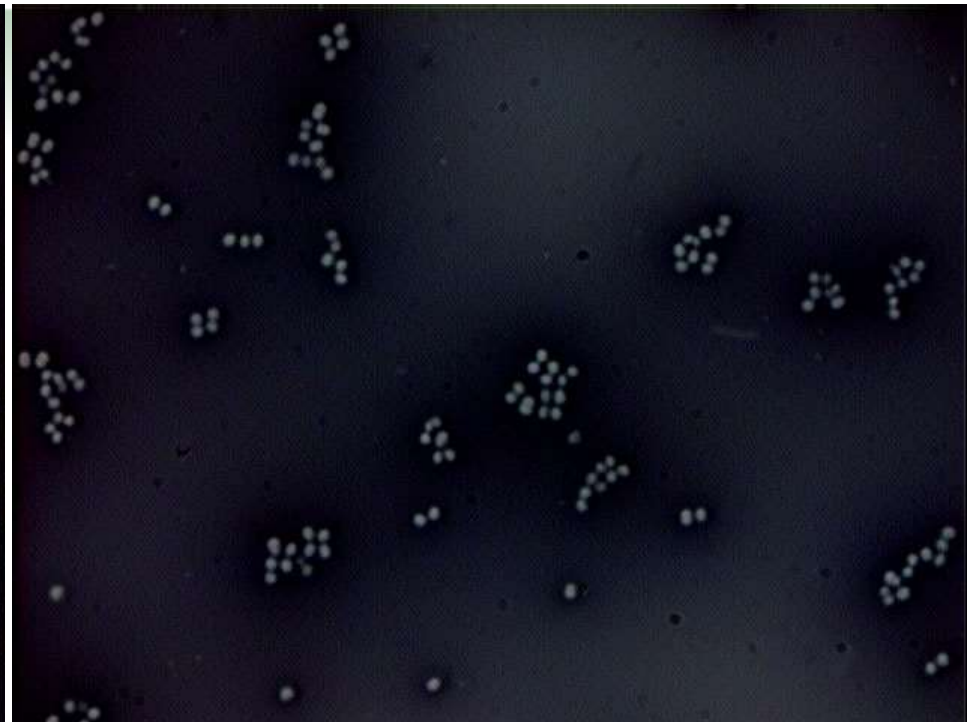


6. Observe the cells with microscope.

RESULT



Negatively Stained *Bacillus*



Negatively Stained Cocci

أشروق الشهراني




Purpose: To recognize the three basic shapes of bacterial cells.



Principle: In order to observe most bacterial cells using bright field microscopy the cells must be dark enough to see, that is they must have contrast to the light. To create contrast a simple stain can be used. Simple stains use basic dyes which are positively charged. These positive dyes interact with the slightly negatively charged bacterial cell wall thus lending the color of the dye to the cell wall.





The simple stain can be used to determine cell shape, size, and arrangement. ©

True to its name, the simple stain is a very simple staining procedure involving only one stain. You may choose from methylene blue, Gram safranin, and Gram crystal violet. ©

Basic stains, such as methylene blue, Gram safranin, or Gram crystal violet are useful for staining most bacteria. These stains will readily give up a hydroxide ion or accept a hydrogen ion, which leaves the stain positively charged. Since the surface of most bacterial cells is negatively charged, these positively charged stains adhere readily to the cell surface. For more detailed information, consult page 93 of your lab manual. ©

