



140 MBIO

# LAB 6: SIMPLE STAINING + NEGATIVE STAINING

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# PURPOSE

- 1. To recognize the shape of bacterial cells.**
- 2. To determine the arrangement 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



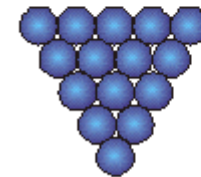
diplococcus



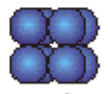
streptococcus



tetrad



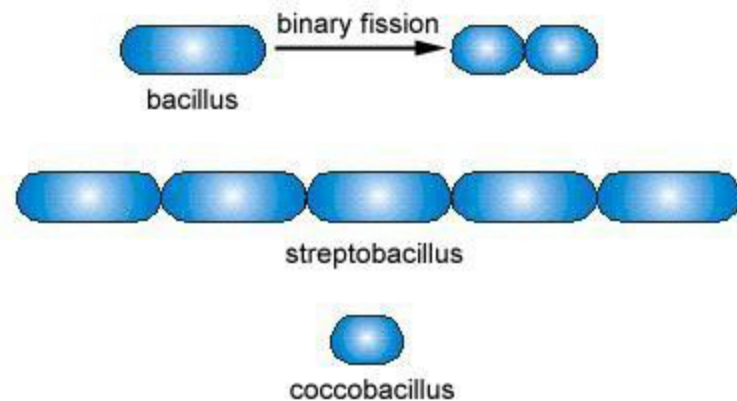
staphylococcus



sarcina

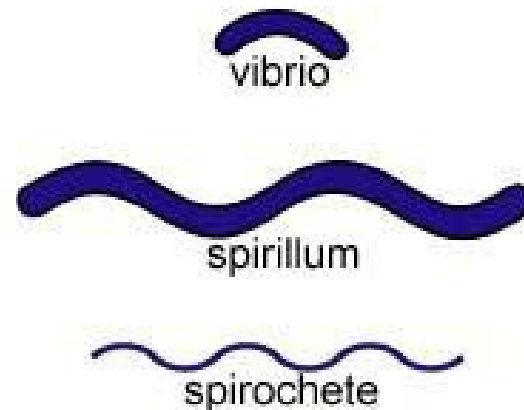
## 2- Bacillus

- ① **Bacillus:** a single bacillus
- ① **Streptobacillus:** bacilli in chains
- ① **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



# Various SHAPES OF BACTERIAL CELLS



Coccus



Coccobacillus



Vibrio



Bacillus



Spirillum



Spirochete



# SIMPLE STAINING





# SIMPLE STAINING:

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

# SIMPLE STAINING:

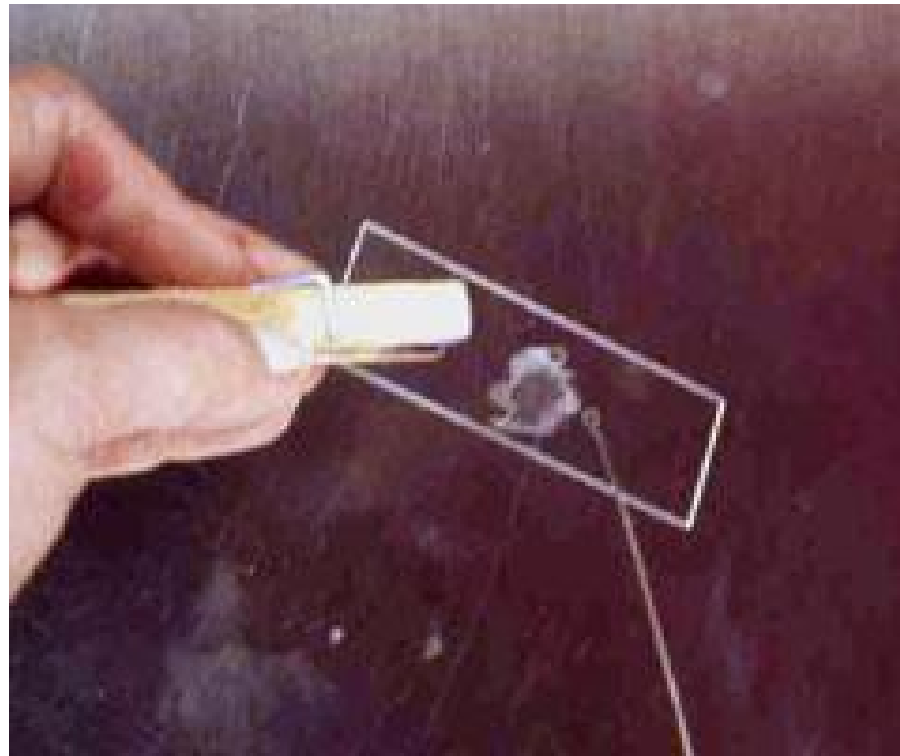


## 1. Prepare the bacterial smear.

- 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 (1Cm<sup>2</sup>).

# SIMPLE STAINING:

**2. Let the smear air dry completely.**



2017 -18

# SIMPLE STAINING:



## 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 STAINING:



## 4. **Stain the smear.**

- ③ Place the slide on a rack over the sink. Using drops cover the smear with stain and leave it for 60-90 seconds. Rinse gently and blot dry (we press a filter paper gently to remove any drops of water).

# SIMPLE STAINING:



5. Place the slide on the stage of light microscope. Choose the correct field using the lower power object lenses (4X and 10X ) by coarse adjustment to bring the slide nearer toward lenses. After that, examine the microscopic field using the 40X object lens. Keep the stage where it is, then, place a drop of oil directly on the stained smear .Turn the oil lens(100x) into position and fine focus to observe the cells.



# RESULTS

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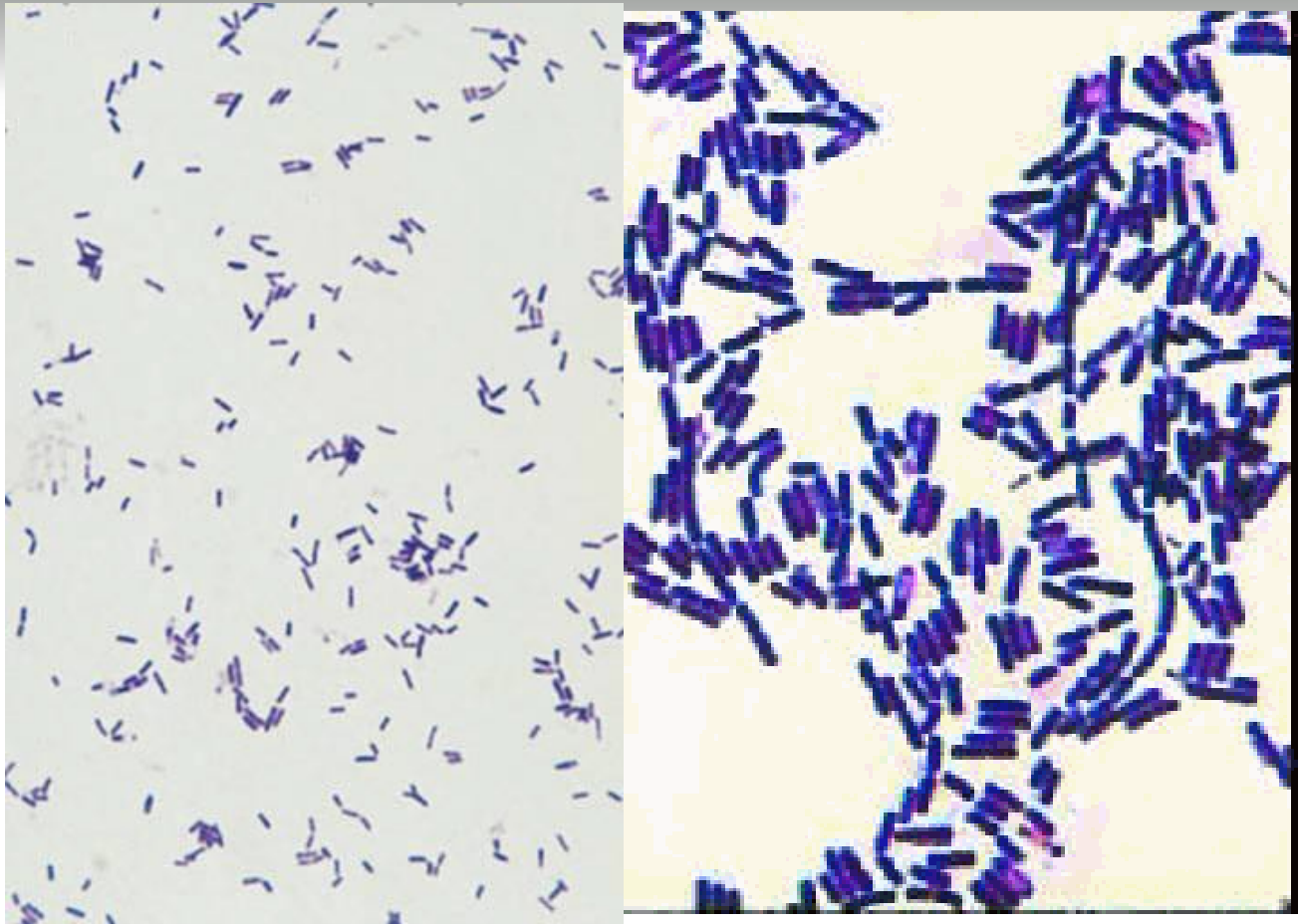
15

# Coccus (cocci pl.)

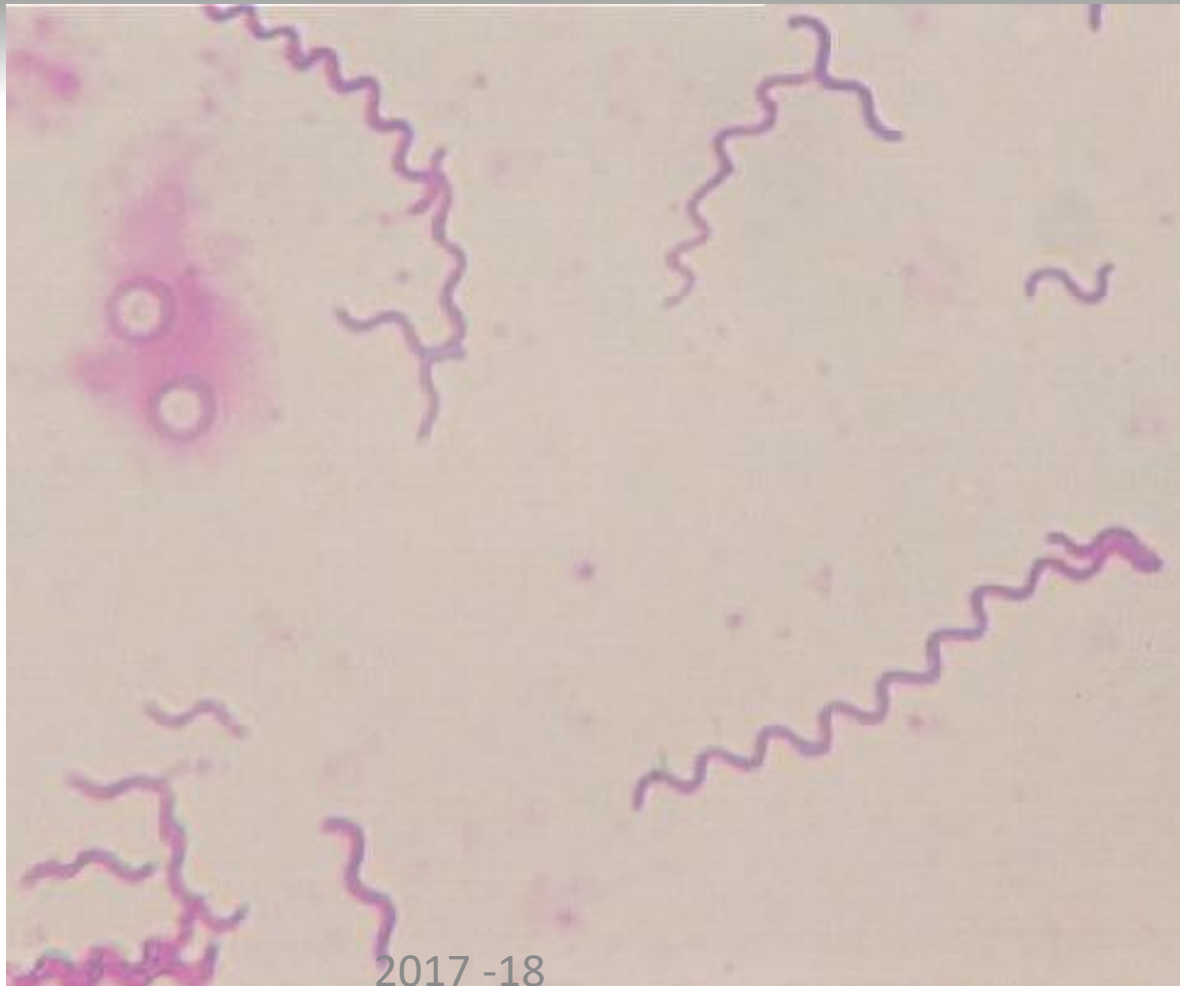




# Bacillus (Bacilli pl.)



# Spirillum (Spirilli pl.)



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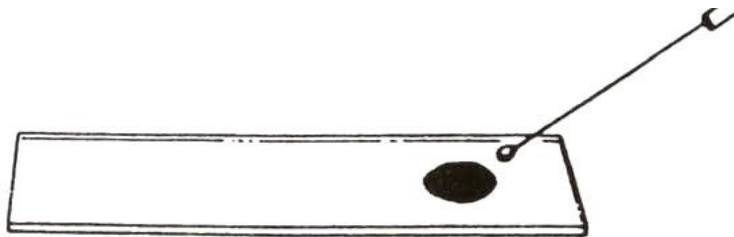
# NEGATIVE STAINING

It is the staining of the background of the bacterial smear while the cells stay colourless!

# NEGATIVE STAINING

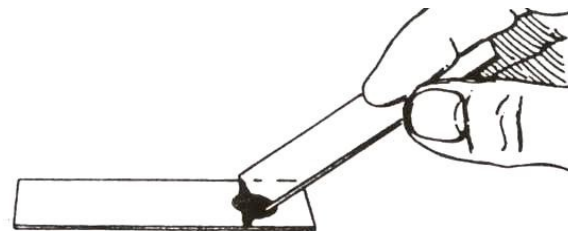
1. Place a very small drop of **nigrosin** near one end of a slide.

2. Remove a small amount of the culture with an inoculating loop and disperse it in the drop of stain .

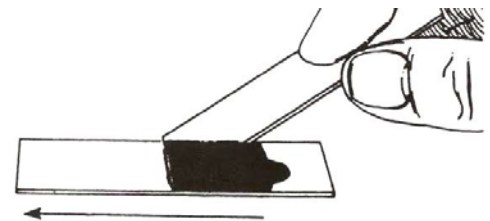


# NEGATIVE STAINING

3. Use another clean slide to spread the drop of stain containing the organism using the following technique. Then, get rid of the slide or keep it until dry completely (you may use a slide cover instead).



**1**



**2**

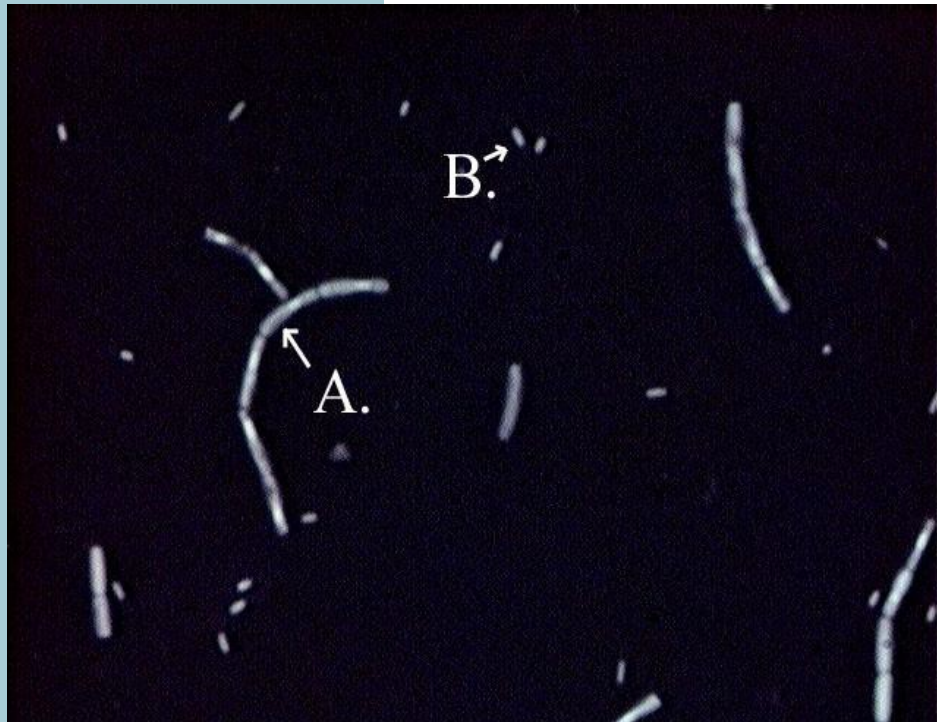
# NEGATIVE STAINING

5. Allow the smear to dry without heating.

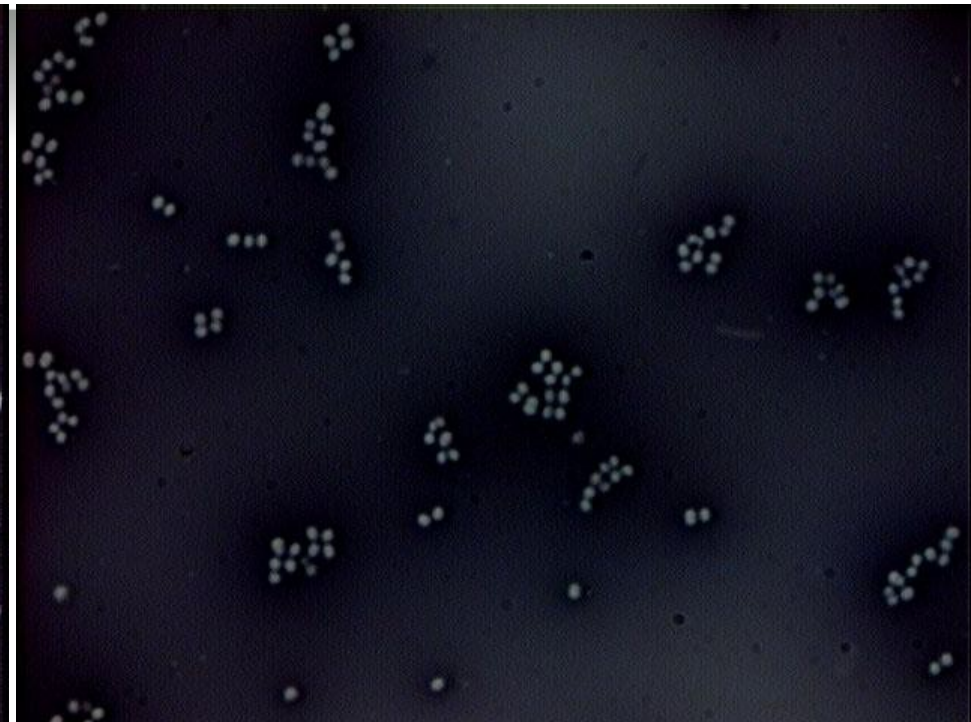


6. Observe the cells under the microscope.

# RESULTS



Negatively Stained *Bacillus*



Negatively Stained Cocci

# VIDEO TIME







# THANK YOU

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