

# **Microbiology Laboratory 5**

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## **Observations from Last Week's Lab**

### Aseptic technique

- Examine your broth culture and slant.  
If the broth culture is turbid you have growth.  
Can you observe growth on your slant?

One often repeated error when there is no growth is that the student did not let the loop cool enough before beginning the transfer.

Can you tell if only one organism (colony morphology) is present?  
Can you tell if there is contamination?

- Place your broth culture and slant in the rack to be autoclaved.

### Spread plate

- Remember how the Snyder Test Agar is selective ( $\downarrow$  pH) and differential (producing yellow color in agar when the bacteria can ferment glucose into lactic acid).
- Examine your plate.  
Do you have cultures with yellow around them?  
Note: You are not looking at the color of the colony, you want to look at the color of the agar.

Based on your observations, are you at risk for cavities?

If your plate did not change the color of the agar make sure you see another student's plate that has resulted in yellowing the agar.

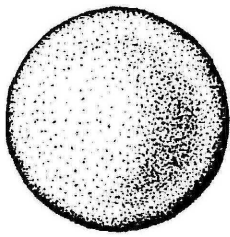
- Place your plate in the bin to be autoclaved.

### Streak plate

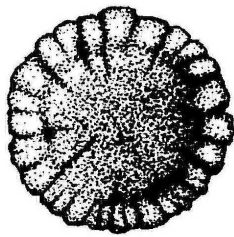
- Examine your streak plate.  
Do you have isolated colonies (colonies that are not touching any others)?  
Can you distinguish more than one colony type? (Hopefully not.)
- If you did not obtain any isolated colonies streak another plate from your current plate for isolation and incubate at  $37^{\circ}\text{C}$  for next week.
- Use the key on the next page to determine the colony characteristics of your bacteria. Record on the chalk board your observations for the class.

# Colony Characteristics

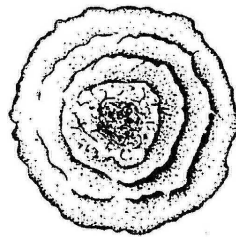
## Configurations



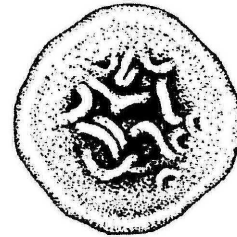
Round



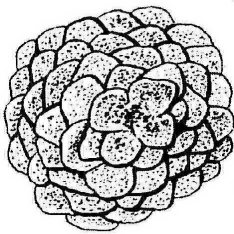
Scalloped



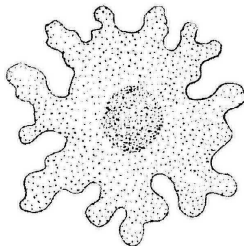
Concentric



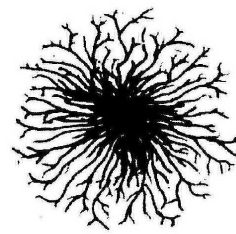
Wrinkled



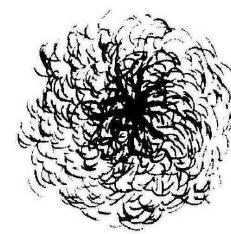
Complex



Irregular



Filamentous



Rhizoid

## Margins



Smooth



Wavy



Lobate



Irregular



Ciliate



Branching



Wooly



Thread-Like



Hair-Like

## Elevations



Flat



Raised



Convex



Drop-Like



Umbonate



Hilly



Ingrowing  
into medium



Crateriform

Describe your Culture:

Organism Name \_\_\_\_\_

Configuration \_\_\_\_\_

Margin \_\_\_\_\_

Elevation \_\_\_\_\_

Color \_\_\_\_\_

## Gram Stain

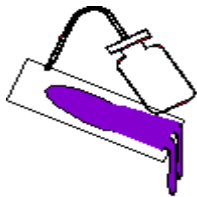
In 1882 A Danish physician by the name of Christian Gram was working in Berlin. He was trying to develop a method to differentiate bacteria from eukaryotic cell nuclei in stained lung tissue samples from people who died of pneumonia. While Dr. Gram was unsuccessful in his quest, he did develop the most used staining technique in microbiology. He published in 1884. The Gram stain (as it became known) differentiates between two large groups of bacteria called Gram positive (+) and Gram negative (-). Thus the Gram stain is a differential stain and is usually the first step done when identifying an unknown bacteria. The Gram stain basically consists of three steps: stain, decolorize, and counter stain.

First make a thin smear using a culture that is only 16-18 hours old. Then heat fix your slide.

### Gram Stain Procedure



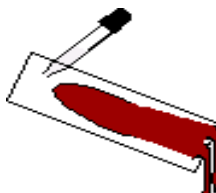
- **Primary stain** - flood your smear with **crystal violet** for 20 seconds. This will stain every cell a dark purple.



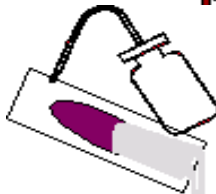
- Wash - rinse off the crystal violet with water for 2 seconds.



- **Mordant** - flood the smear with **Gram's iodine** for 1 minute. The iodine does not stain anything. The mordant complexes with the crystal violet creating an insoluble large complex that can be trapped within the cell. At this point all cells still appear a dark purple.



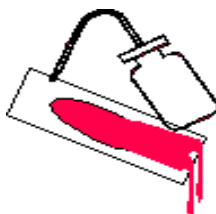
- **Decolorize** - Wash the smear with alcohol until no more color runs from the smear. This is the critical step in the process. Stop not when the smear loses all its color (may or may not happen) but stop when no color is running off the smear.



- Wash - rinse off the alcohol with water for 2 seconds to stop the alcohol reaction.



- **Counter stain** - flood the smear with **Safranin** for 1 minute. Since all Gram - bacteria are colorless we need to stain them to see them. Safranin is a red stain to distinguish it from the purple crystal violet. Thus Gram - bacteria appear a reddish pink color.



- Wash - finally rinse the smear with water for 2 seconds to wash away any excess safranin.
- Blot dry your slide and examine it under the microscope.

## Gram Stain Results

Report the results of your Gram stain on the chalk board with the name of your organism.

### Gram Stain Explanation

There are basically two types of bacteria, Gram + and Gram -. Gram + bacteria have a very thick peptidoglycan cell wall. During the decolorization step the alcohol collapses (dehydrates & shrinks) the thick peptidoglycan layer which traps the crystal violet - iodine inside the cell. Thus Gram + bacteria appear purple in the end. If you over decolorize it is possible to wash out the normally trapped crystal violet - iodine from inside the cell. That is why the decolorization step is critical. Note the Gram + bacteria are also stained by the safranin but the crystal violet over powers the lighter colored safranin so they still appear a dark purple color.

Gram - bacteria have a thin peptidoglycan wall and a second phospholipid bilayer (called the outer membrane). When the small amount of peptidoglycan is collapsed by the alcohol it is not thick enough to trap the crystal violet - iodine inside the cell so it washes out. If you don't wash long enough Gram - bacteria will retain some crystal violet - iodine and will appear purple. After decolorization the Gram - bacteria appear colorless so they must be stained to observe them. Since safranin is used, which is red, Gram - bacteria appear red in color.

Note: Some Gram-positive bacteria may lose the stain easily and therefore appear as a mixture of Gram-positive and Gram-negative bacteria (Gram-variable). Older cultures often stain as Gram variable. That is why it is important to stain a fresh culture that is only 16-18 hours old.

Color changes that occur at each step during Gram staining

<u>Reagent</u>	<u>Gram Positive</u>	<u>Gram Negative</u>
None (heat-fixed cells)		
Crystal violet (20 sec.)		
Gram's Iodine (1 min.)		
alcohol (10-20 sec) (till no color runs)		
Safranin (1 min.)		