LAB 3
CHARACTERIZING YOUR "UNKNOWN" BACTERIA
AND USING MORE COMPLEX STAINS

Objectives

In this lab you will learn how to:

- describe bacteria on the basis of colony and cell morphology
- isolate bacterial colonies onto agar slants using aseptic techniques
- stain bacteria using several different methods
- stain your own plaque/epithelial cell sample

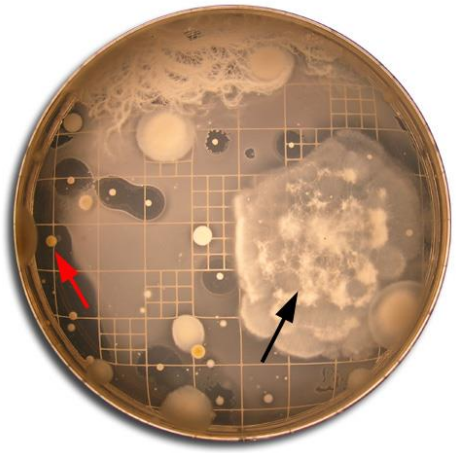
Part I: Isolating Your Unknown Bacteria and Describing Colony Morphology

Last week you inoculated Petri plates with bacteria collected from your skin. Now that they have had a chance to grow, you will examine the colonies in more detail, and characterize them according to their **colony morphology** (appearance of colony). You will also sample one colony that you collected from your skin, and transfer it onto an agar slant using aseptic techniques.

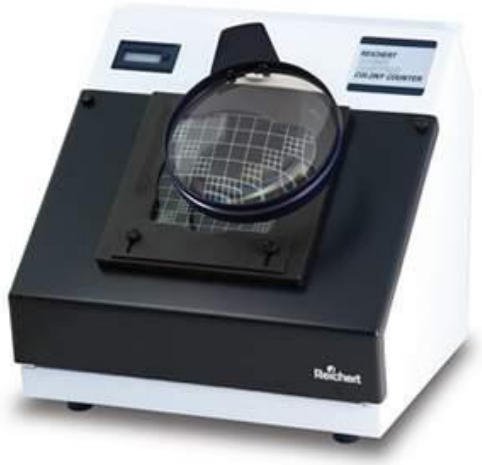
Procedures

Observe the colonies growing on the Petri plate that contains your skin sample. There should be a variety of colonies in terms of color, size, shape, and texture (for example, slimy white or yellow dots of bacteria, and fuzzy black or gray colonies of fungi).

Ignore the fungi! Using the **Quebec colony counter** (a light box with a magnifying lens to closely observe and count the colonies) or your naked eye, choose a bacterial colony that is all by itself, away from any other colonies. Draw a circle on the bottom of the Petri plate around the colony, so you can keep track of your selected colony.



Examples of bacterial colonies (red arrow) and fungal colonies (black arrow).



The Quebec colony counter.

If no colonies have grown on the Petri plate that contains your sample, or if all of the colonies have grown together (representing contamination of two or more colonies), then use a lab partner's sample.

Examine the bacterial colony from your Petri plate and characterize it by color, size, form, and margin in Table 1.

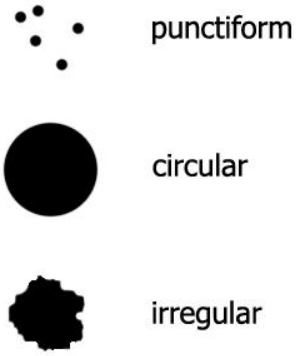
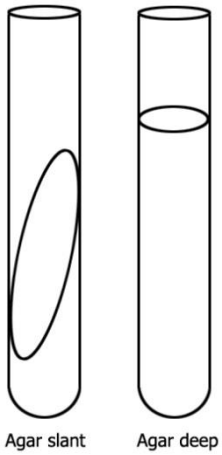
Colony Forms:	**Colony Margins:**
	

Table 1. Your unknown bacteria colony morphology.

color:	
size (mm):	
form:	
margin:	

When you have finished describing colony morphology, transfer some cells from the colony to an agar slant using a sterile transfer loop. An **agar slant** is a tube of nutrient agar medium that has been allowed to cool at an angle, creating a large amount of surface area for the bacteria to grown on, within the small volume of a test tube. Remember that an **agar deep** is a tube of culture medium that is melted and poured into a sterile Petri dish.



Don't forget to label the isolate so that you will know it's yours! For example, label it "skin" or "cell phone" and include your initials. Incubate the agar slant at 37°C.

Part II: Staining Your Unknown Bacteria

In the rest of this lab you will dye bacteria using common staining techniques. Visualization of bacteria can be very difficult without staining them first, because they are tiny and transparent cells otherwise. **Stains** are colored chemicals that bind to cellular structures such as cell walls, lipids, proteins, and nucleic acids.

You have already learned how to conduct a **simple stain**, in Labs 1 and 2. **Differential stains** use two contrasting stains (such as crystal violet and safranin) to separate bacteria into groups (such as Gram-positive or Gram-negative). **Special stains** are used to allow visualization of structures such as capsules, flagella, and endospores.

Procedures

First, you will prepare a slide from your unknown (UK) from the same colony on the Petri plate that you transferred to the agar slant in Part I. After the smear preparation, you will conduct a Gram stain of this sample.

Smear Preparation

Clean your slide well with cleanser, rinse and dry it thoroughly, use a marker to make a small circle on the bottom of the slide. Label each slide on top according to the bacterial culture used.

Flame sterilize the end of the transfer loop. Then place 1 or 2 loopfuls of water in the center of the circle, using the sterile loop. Flame sterilize the end of the transfer loop again. From the bacterial culture on the solid media in your Petri plate, gently scrape a *very small amount* of the colony off the surface of the agar. Just touching the colony is often enough. DO NOT dig into or gouge the agar!

Add the bacteria to the drop of water, and spread the suspension (**emulsify** it to a milky appearance) to fill the circle on the slide. The smear should look like very diluted skim milk. Immediately flame your loop again to kill the bacteria remaining on it.

Allow the small droplet containing bacteria to **dry** (use the slide warmer).

Then, using a clothespin to handle the slide, **heat fix** the bacteria to the slide by waving the slide through the flame 3 times (1 or 2 seconds each pass). You are now ready to Gram stain your sample.

Gram Stain

Cover the air-dried, heat-fixed smear with **crystal violet** and leave for 30 seconds.

Wash the slide carefully with water. Do not get water directly on the smear.

Without drying, cover the smear with **iodine** for 30 seconds.

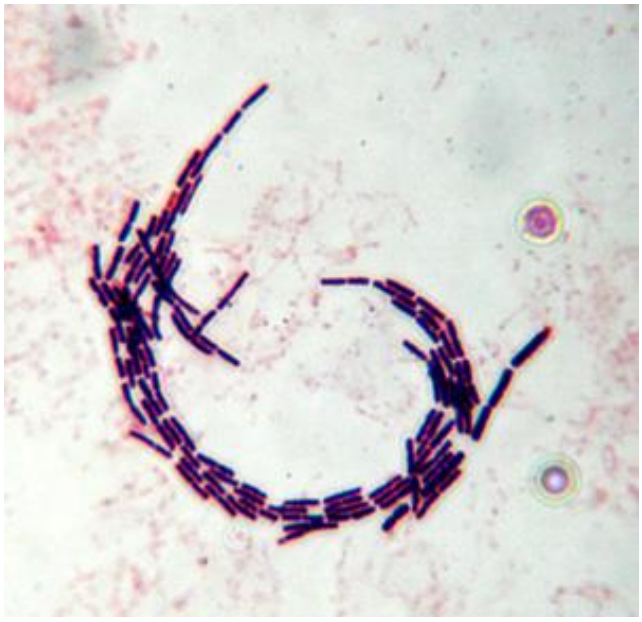
Without washing, decolorize it with **alcohol** (95% ethanol). Let the alcohol run through the smear until no more purple washes out (usually a few seconds). The degree of alcohol decolorizing depends on the thickness of the smear; this is a critical step! Do not over-decolorize. Experience is the only way you will be able to determine how long to decolorize.

Immediately wash the ethanol off with water.

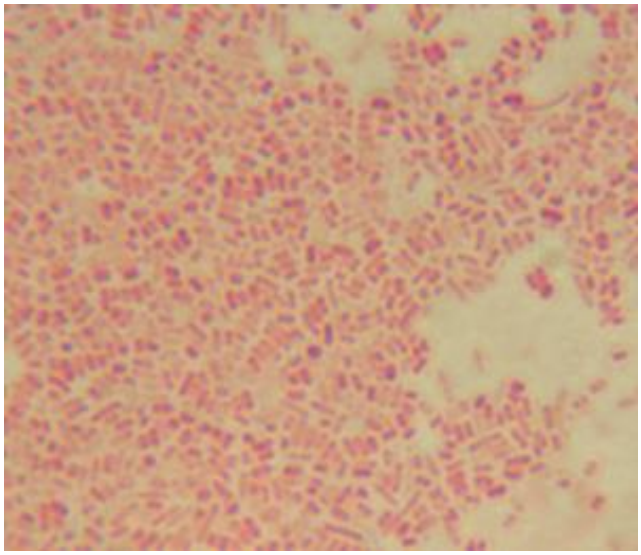
Without drying, cover the smear with **safranin** for 30 seconds.

Wash with tap water and dry the slide on the slide warmer.

Once the slide is completely dry, examine it under low power, then work your way up to 1000X magnification with oil immersion. A **Gram-positive** reaction will appear as PURPLE/BLUE cells and a **Gram-negative** reaction will appear as PINK/RED cells.



***B. cereus*, a Gram-positive bacillus (1000X).**



***E. coli*, a Gram-negative shortened bacillus (1000X).**

Bacterial Cell Morphology:	Draw Your Bacteria Cells Below:
 staphylococci  streptococci diplococci  tetrad  bacilli  endospores  vibrio  spirillum	

Fill out Table 2 (below) with the bacterial cell morphology description of your sample. Include the source of bacteria, the stain reaction, the shape of cells, and arrangement of cells.

Table 2. Your unknown bacteria cell morphology.

BACTERIA SOURCE (arm, cell phone)	STAIN REACTION (Gram + or Gram -)	CELL MORPHOLOGY (staphylococci, streptococci, bacilli, etc.)

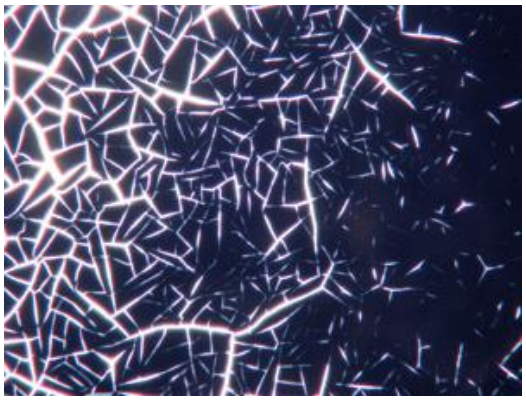
Part III: Other Staining Techniques

Negative Stain

Clean and mark a new slide as discussed before. Sterilize your transfer loop and transfer some *Bacillus cereus* from the broth culture to the slide. Dry the slide and then heat fix it.

Place a small drop of **nigrosin** at one end of the slide. Using the end edge of another slide, spread the drop out to produce a very thin smear. The angle of the spreading slide will determine the thickness of the smear.

Dry the slide again. Do not heat fix it. Examine at low power, then work your way up to 1000X with oil immersion. Ignoring the large white cracks, look for small, white, rod-shaped bacteria standing out against the dark purple background.



Cracks in a negative stain, under low magnification (40X).

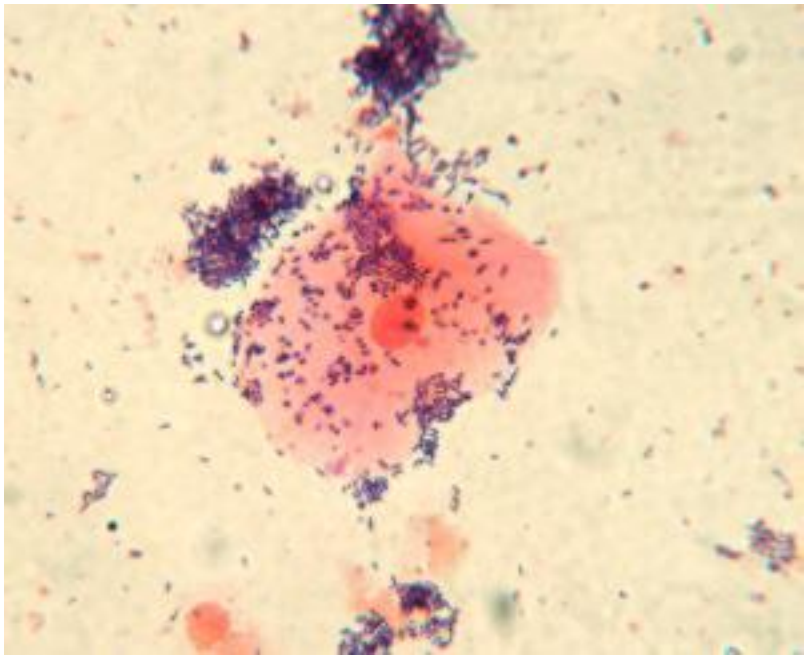
***Bacillus* stained with a negative stain (1000X).**

Part IV: Plaque / Epithelial Cell Stain

Clean and mark a new slide. Flame sterilize the end of the transfer loop. Then place 1 or 2 loopfuls of water in the center of the circle, using the sterile loop. Flame sterilize the end of the transfer loop again.

Transfer some epithelial cells from your mouth to the slide by gently scraping between your teeth and gums, or against your tongue or cheek, with a sterile toothpick. Add the sample to the drop of water and swirl the suspension (emulsify it) to fill the circle on the slide.

Dry the slide on the slide warmer. Then, using a clothespin to handle the slide, heat fix the bacteria to the slide by waving the slide through the flame 3 times (1 or 2 seconds each pass). You are now ready to Gram stain your sample and then examine it carefully under the microscope.



Gram-stained epithelial cell with associated bacteria (1000X).