

LAB 2

COLLECTING AND CULTURING “UNKNOWN” BACTERIA AND PREPARING A GRAM STAIN

Objectives

- In this lab you will learn how to:
 - collect and culture microorganisms from the skin or from your cellphone
 - use aseptic techniques to transfer bacteria
 - conduct a Gram stain of bacteria

Introduction

Microbes are everywhere, and can be easily collected from air, water, food, soil, and your own body. Microbiologists routinely face the challenge of collecting and isolating pure cultures of these microbes for study. Once collected, the microbes can be grown on a **culture medium**, a liquid or solid that contains the nutrients necessary for microbial survival and growth. The culture medium must first be sterilized, then inoculated with the microbe and incubated at the appropriate temperature for a certain amount of time (usually 24-48 hours). Microbes collected from your body will grow best at human body temperature, or 37°C (98.6°F).

Solid culture media require the addition of a solidifying agent, such as **agar**. Agar is a polysaccharide derived from marine algae. Most bacteria cannot digest it, which is a good thing because otherwise, the medium would be liquified as the bacteria digested it! Most solid culture media, such as **nutrient agar**, contain 1.5% agar in addition to various nutrients. Because agar liquifies at 100°C (212°F) and resolidifies at 40°C (104°F), an **agar deep** (a tube of previously-prepared culture medium) can be melted and poured into an empty, sterile **Petri dish**. A Petri dish holds 15-20mL of medium and provides a large surface area for bacterial growth and isolation. Once in the Petri dish, the medium will solidify as it cools and remain solid at common incubation temperatures.

On a **Petri plate** (a Petri dish filled with culture medium), bacteria will grow and multiply to form colonies. A bacterial colony often looks like a small dot or streak on the medium, and contains *millions* of individual cells. A single species of bacteria growing in culture medium is considered a **pure culture**.

Part I: Pouring Petri Plates and Inoculating the Culture Medium

Each person will make a Petri plate to collect a bacterial sample from their skin or cellphone. Make sure you know which side of the empty Petri dish is “up,” before pouring the melted medium into it!

Procedures

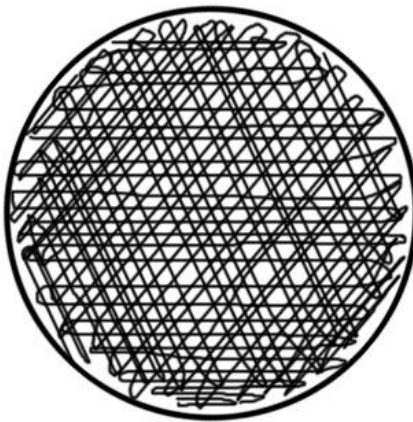
Obtain:

- melted nutrient agar deep (from the beaker on the hot plate)
- sterile Petri dish
- sterile disposable swab

Handle the melted nutrient agar deep carefully, because it can burn you! Use a test tube clamp to handle the hot tube. You might want to put the hot tube into an empty aluminum can, then take it back to your desk, along with a Petri dish and a disposable swab.

Label the **BOTTOM** of the Petri dish with your initials. Flip it right-side up, open the top and pour the melted agar into it. Let it cool (this should take 15 minutes, or until the medium looks slightly translucent). To keep unwanted microbes out, always **keep the lid on**, unless you are inoculating the Petri plate or sampling from it.

Once it has cooled, you are ready to sample some bacteria from your skin or cellphone. Remove the sterile swab from its wrapper, streak it across your skin somewhere on your body, or across your cellphone screen. Next, streak the swab on the surface of the agar, back and forth in a zig-zag pattern, as shown below:



Incubate this plate upside down for 48 hours at 37°C (98.6°F).

Why do you suppose the Petri plate should be inverted when it is in the incubator?

Why is it being incubated at 37°C (98.6°F)?

Part II: Aseptic Transfer of Bacteria and Gram Staining

Next you will make a slide of two common laboratory bacteria (*E. coli* and *Bacillus megaterium*), Gram stain it, and examine it under the microscope.

Procedures

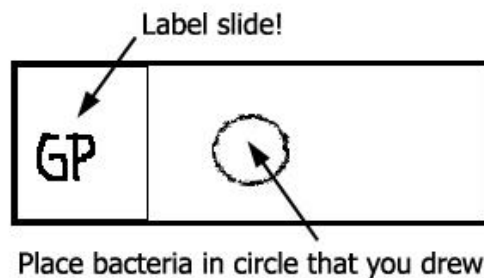
Obtain:

- tube of bacteria (EC = *E. coli*)
- tube of bacteria (BM = *Bacillus megaterium*)
- new, cleaned slide
- transfer loop
- burner
- striker to light the burner
- clothespin

Clean your slide well, then rinse and dry it thoroughly. Mark a small circle on the slide with a marker – this is where you will put the bacteria. Actually, the side that is marked will be the bottom of the slide, so put the bacteria on the other side (top) of the slide. Don't forget to label each slide with your initials.

Next, gently swirl the tube of EC bacteria to suspend the cells. **Flame sterilize** the end of the transfer loop as demonstrated by your instructor. Allow the transfer loop to cool down (a few seconds), without contaminating or touching the loop to any surface, such as the desktop. Open the cap of the tube with the EC bacteria in it, dip the loop in, and transfer a small droplet of EC bacteria to the slide. Your instructor will demonstrate the right way to do this.

Immediately flame sterilize the transfer loop again, without contaminating or touching the loop to any surface, such as the desktop! Only then can you set the transfer loop down on your desk.



Repeat the process with some BM bacteria, adding it to the drop of EC bacteria on the slide.

Allow the drop containing EC and BM bacteria to **dry** on the slide with the slide warmer.

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).

Next, follow these steps to Gram stain your bacterial 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.

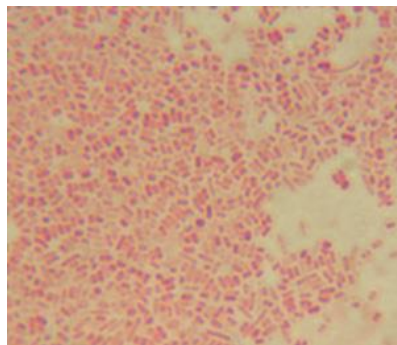
Immediately wash the ethanol off with water.

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 (there is no coverslip!), then work your way up to 1000X magnification with oil immersion.

A **Gram-negative** reaction will appear as PINK/RED cells and a **Gram-positive** reaction will appear as PURPLE/BLUE cells.



Gram-negative *E. coli* (left) and Gram-positive *Bacillus megaterium* (right).