

# SWAB, SPREAD, AND STAIN

## A MINI-MICROBIOLOGY LAB

### Objectives

- 1) Apply proper aseptic techniques when handling bacterial samples.
- 2) Obtain and spread bacterial samples onto a growth medium.
- 3) Prepare and stain bacterial smears and use an oil immersion lens to view bacterial cells.

### Introduction

Bacterial cells are typically transparent so it can be difficult to distinguish their details under a light microscope. To enhance the contrast between the bacterial cells and the bright background produced by the microscope, bacterial cells are smeared onto a microscope slide and stained. Stains such as gram's crystal violet and gram's safranin are used because they adhere nicely to the bacterial membrane and can help differentiate between gram+ and gram- bacteria. In a typical microbiology course you would apply many different staining techniques to aid in identifying and distinguishing between bacterial species. In this lab, you're just going to apply a simple staining technique so you can become familiar with the basic technique.

For this lab you are going to basically do three things: (1) **Swab** an area where you think bacteria are growing, (2) **Spread** the stuff you collected onto a culture medium to see if anything grows, (3) Prepare and **stain** a smear of bacterial cells so they can be observed under a light microscope.

### Materials

- 1) Sterile Q-tip
- 2) Test tube
- 3) Bottle of distilled water
- 4) Agar plate
- 5) Sharpie marker
- 6) Inoculating loop
- 7) Microscope Slide
- 8) Bunsen burner
- 9) Clothes pin
- 10) Methylene blue stain
- 11) Absorbent paper
- 12) Gloves

## Procedure

### *Obtain Bacterial Sample*

- 1) Make a prediction about where you think bacteria are growing. Write your name and prediction on the handout provided. Be specific; don't just say 'on the sink' instead say something like "on the cold water knob."
- 2) Obtain a sterile Q-tip and a test tube. Add a few drops of distilled water to the test tube. Use the sterile Q-tip to swab the area of interest, then dip the Q-tip into the test tube and swirl it around in the water. Leave the Q-tip in the test tube until you've gathered the materials necessary to spread your bacteria sample on the growth medium.

### *Spread Bacterial Sample*

- 3) Obtain a growth agar plate. Write your name, section number, and date on the back of the plate (not the lid) using a sharpie maker.
- 4) Swirl your Q-tip in the test tube once more and gently tap it against the inside of the test tube to drain excess water.
- 5) Spread the bacteria sample smoothly across the entire surface of the plate. This technique is not the same as what's called the streak-plate technique, since here our goal is to maximize growth on the agar plate. In the streak-plate technique the goal is to isolate individual colonies.
- 6) Close the lid on the plate and turn it upside down. Do you know why the agar plate should be flipped upside down? Ask your TA where to put your plate.
- 7) After you leave lab, your TA will place your plates into an incubator at 37<sup>0</sup>C. Two days later, your plates will be removed from the incubator and placed in the freezer until you return to lab the following week.

### *Observe Bacterial Sample*

- 8) Observe your agar plate. If it doesn't contain any growth, then your experiment is finished and you need to partner with someone that has growth, so you can observe the techniques discussed in the remaining steps.

### *Prepare a Bacterial Smear*

- 9) Obtain a clean microscope slide. How do you know if the microscope slide is clean? You know it's clean when you can place a drop of water on the slide and the water spreads evenly across the slide. If the water beads, then there's probably some oil residues on the slide (most likely from your fingerprint) and it most likely also contains bacteria [Note: potential source of contamination].

In this case it really doesn't matter because we're going to apply a flame to the slide anyways.

- 10) Apply one drop of distilled water to the center of the slide. Put the slide down on the counter.
- 11) Setup a burner with a low flame. Sterilize your inoculating loop in the flame. Let the loop cool for 30 seconds. Don't blow on the loop [Note: another potential source of contamination].
- 12) Use the sterile inoculating loop to scrape some bacteria off your plate. You don't need a huge glob, just a little at the end of the loop.
- 13) Swirl the loop + bacteria around in the drop of water you placed on the slide. The water should change from clear to cloudy.
- 14) Attach the clothes pin to the long side of the microscope slide and pass the slide over the burner flame until the water evaporates. If you hold the slide over the burner two bad things will happen. First, you'll crack the slide or it will explode. Second, the bacterial cells will cook and their structures will be indistinguishable. Passing the slide over the top of the flame 3-5 times should be plenty. Hold your slide using the clothes pin not your hands for about 30 seconds, then set it down on the counter. Turn off the burner.

#### *Stain the Bacterial Smear*

- 15) With the clothes pin still attached to your slide, hold the slide over the sink and place one drop of methylene blue stain on the smear. Let the stain sit for one minute then pour off excess stain and rinse with distilled water. Don't squirt water directly at the smear or it might wash off, instead squirt around the edges of the smear.
- 16) Remove excess water from the slide by blotting the slide dry with absorbent paper (Note: DO NOT RUB and do not use paper towels).
- 17) Examine the stained smears under the light microscope at different magnification powers including the oil immersion lens.
- 18) Draw what is seen in the field of view using the oil immersion lens.
- 19) Clean up your work area and dispose of gloves and slides in Biohazard bag.

#### Assessments

Objective 1: Each student will wear gloves, use sterile tools, and maintain a clean and safe work station.

Objective 2: Each student will inoculate an agar plate with bacteria and properly label the plate with the required information.

Objective 3: Each student will successfully use the oil immersion lense technique so they can view and draw what is seen under the microscope.