Project 6: Throat Cultures and Identification

Readings:

- https://labtestsonline.org/lab/photo/throat1/start/3

Identification of Gram-Positive & Gram-Negative Bacteria

- Guide to laboratory stock cultures developed during Lab Project 4

Purpose:

The purpose of this experiment is for students to understand microbe identification in the context of a clinical situation. This lab uses the clinical simulation of the throat culture to more precisely define the process of determining the genus and species identification of a potential pathogen from a clinical specimen.

Outcomes:

After you complete this lab, you will be able to:

- Isolate and identify bacteria in an unknown sample.
  - Perform an isolation streak plate.
  - Make decisions about the type of tests that must be performed to confirm the genus and species of an unknown bacterium using the methods of colony morphology (using TSY), differential staining (Gram, Acid-fast, Endospore), differential and selective media (blood agar, mannitol salt, MacConkeys), as well as more specific laboratory tests.
  - Perform the tests, collect and interpret the data.
- Perform and interpret antibiotic sensitivity testing.
  - Identify the time necessary for performing the test.
  - Identify limitations of sensitivity testing.
  - Relate microbial structure to mode of anti-microbial action.

Terms to Know:

Be able to define these terms and apply them in the laboratory.

Hemolysis		
antibiotic sensitivity	Enterobacteriaceae	selective media
	Enterococcus	
Bacitracin sensitivity	MacConkey's medium	sterile specimen
colony forming unit	medical technologist	
differential media	mode of action	viable cell counts

Throat Culture & Identification – SESSION 1

INTRODUCTION:

Recall from the Lab Project 4 *Introduction*, that our bodies are composed of 100 trillion (10^{14}) cells. We are inhabited by a quadrillion (10^{15}) bacterial cells, known as **normal flora**. Normal flora is found on any exposed surface of the body (i.e., skin and mucus membranes), but healthy internal organs are sterile – no bacteria live there.

You are colonized at birth. Most of your normal flora are long-term inhabitants. Others, called transient flora, are just passing through.

Our normal reaction to the notion that we are covered in bacteria is “YUCK!!” However, with all that bacteria do for us, this is a misguided reaction. Normal flora take up space (preventing pathogens from moving in and setting up housekeeping), consume the scant nutrients available (again, competing with potential pathogens) and put out waste products that keep other less desirable microbes from setting up shop. In this lab you will investigate the normal-flora microorganisms that inhabit the different surfaces of your body.

Normal flora must be considered when examining cultures for a suspected pathogen. Knowing the types and locations of various normal flora may assist clinicians in deciding whether a reported microbial suspect is friend or foe. When performing throat cultures, the major groups of bacteria that may cause infection could easily be overrun by normal flora. On the Petri plate we need some mechanism to distinguish normal oral flora from potential pathogen.

Also from Lab Project 4 you read that blood agar is ***differential***, and microbiologists can easily distinguish some clinically significant bacteria by their appearance, or hemolysis pattern, when cultured on blood agar (Bauman p. 180, Fig. 6.12). Blood agar contains 5% sheep’s blood. Certain bacteria produce extracellular enzymes called **hemolysins** that lyse the red cells completely (**beta-hemolysis**), producing a clear zone around the colony. Other bacteria produce hemolysins that produce a greenish discoloration around the colony – indicating incomplete hemolysis (**alpha-hemolysis**). Still other bacteria have no effect on the red cells (**gamma-hemolysis**). Blood agar is often inoculated from a patient’s throat swab. (This swab is an example of a **clinical sample**. A clinical sample is a sample of a tissue taken from a patient for diagnostic purposes.) The microbiologist is trying to detect the presence of group A beta-hemolytic streptococci (Gram-positive cocci that cause beta-hemolysis on blood agar). The major human pathogen in this group is *Streptococcus pyogenes,* the causative agent of strep throat. Normal throat flora will exhibit alpha- or gamma-hemolysis, but not beta-hemolysis.

While *Streptococcus pyogenes* is the major pathogen in the throat there may be others. Use your knowledge of the bacterial stock cultures to determine if the bacteria you isolate and identify should be a problem. **For the purposes of this exercise, *Staphylococcus aureus* will be considered a pathogen in the throat.**

Throat Culture

Culturettes

When a patient has a sore throat for which they have sought medical attention, it is common to perform a throat culture. The culturette is a specialized cotton swab with transport medium that is used to swab the back of the patient's throat. It should transported to the laboratory as quickly as possible to avoid any loss of potential pathogen and to prevent normal flora from overgrowing. The culturette is used to swab the first quadrant of an isolation streak plate on blood agar. After this first quadrant streak, the culturette may be discarded in biohazard. Use the inoculation loop and the bactoincinerator to complete the four quadrant isolation streak plate and ready the culture for incubation.

PROCEDURES:

Throat Cultures & Bacterial Identification

1. Obtain a culturette of an 'unknown' from your instructor. Record the unknown identification number / patient name.

2. **Blood Agar** (serpentine streak):
   a. Two students will be using a one Blood Agar plate. Draw a line down the center on the bottom side of plate (where you do all of your labeling).
   b. Each student will take their culturette and make a serpentine pattern in their Blood agar section. See illustration on board for how to make this pattern.

3. **TSY Agar** (isolation streak):
   a. Use the same culturette to inoculate the first quadrant of an isolation streak plate you will be doing on TSY agar.
   b. Discard the culturette in the biohazard container.
   c. Then, sterilize the inoculating loop in the bactoincinerator, allow the loop to cool, and inoculate quadrant 2 of the TSY isolation streak plate.
   d. Continue the isolation streak of quadrants 3 and 4.

4. Ready the plates for storage in the green 'save' bin and it will be incubated at 37^{o} C for 24 hours.

Throat Culture & Identification – SESSION 2

PROCEDURE:

Throat Culture Identification

1. Obtain the throat culture plates set up in Session 1.
2. Record observations of the type (colony morphology) and number of colonies you observe.
3. Record information on the hemolysis patterns you observe.
4. Determine if a 'work up' of a potential pathogen from the throat culture is necessary. If the observed colonies satisfy either of the following criteria, complete the work up. If neither criteria is met report out "Normal Flora; no pathogens observed" in your lab report and do not complete the antibiotic sensitivity.
   a. **Is there a predominate colony type that seems to be the overwhelming growth on the media plate?**
   b. **Do you observe any beta hemolytic bacterial colonies?**
5. Work up of a potential pathogen from throat culture.
   a. Recall that the first step in identification of an unknown is the Gram stain. Complete this test, record your data, and interpret the results. You will need to include these data in your Patient Work-Up form, including pictures. Record your observations (i.e. purple, round, etc.) and interpretations (Gram positive, cocci) in the separate columns provided.
   b. Using your Gram stain as a guide, determine the identification tests you need to set up for identification of the bacterium. The dichotomous key and previous laboratory experience provides the basis for this decision-making. Acquire the necessary media/reagents, set up the tests. Place the tests in the appropriately labeled green incubation bin for incubation and storage until next week.

Some decision making guides are as follows:

- Use positive test results as your definitive test (e.g "I see endospores therefore, given the known laboratory stock bacteria, I know I have identified *Bacillus subtilis"*) rather than a negative test (e.g. "I do not see endospores on the endospore stain and the only other possible Gram positive bacilli is *Mycobacterium smegmatis*").
- Set up identifying tests AND the antibiotic sensitivity tests (procedure below) during Session 2 of the lab. Read all of your results during Session 3.
- Record your OBSERVATIONS and your INTERPRETATIONS in your Patient Work-up Form).
- Identify your unknown (genus and species identification).

Throat Culture

NOTE: If you set up a test that is not needed, you will be charged (penalty points will be deducted from your work-up) for excess use of materials. If the work-up is not completed in a timely manner because you did not set up appropriate tests in the correct order, you will be charged with an ethics violation (penalty points assessed) because you caused a patient to suffer due to improper decision making. You have a classroom full of colleagues. Use this fertile ground for discussion of what you plan to do and why you think it should be done this way.

Antibiotic Sensitivity Testing Procedure

Setup antibiotic sensitivity test using the following modified Kirby Bauer technique:

1 Obtain 3 TSY plates. Two are for testing antibiotic sensitivity. One will be used as a **heat sink**.

2. Identify several well-isolated colonies from a pure culture isolation streak plate of your unknown.
    a. Pick these colonies using a sterile cotton swab.
    b. Transfer the colonies to a tube of 10 mL of sterile saline. Squeegee the cotton swab on the side of the test tube to dislodge the bacterial colonies and make a cloudy suspension.
    c. Discard the cotton swab in the biohazard bag.

3. Use a Pasteur pipette to mix your suspension.

4. Place 5 drops of your bacterial suspension on each of your TSY plates.

5. Using a spreader, spread the bacteria over the surface of the 2 TSY plates you will be using for antibiotic testing.
    a. Pour enough ethanol into a beaker so that the end of the spreader is immersed in the alcohol when dipped.
    b. Dip your spreader in alcohol, and then flame. Be sure there is no paper on the lab bench near the Bunsen burner, so if the flaming alcohol drips, you do not light it on fire.
    c. Wait until no flame is visible, and then touch the flamed spreader to the surface of your heat sink to cool it. You are using a heat sink to disperse the heat from flaming from the spreader more rapidly than if you waited for it to cool in air.
    d. Use the spreader to spread the bacteria on the two plates you will use for antibiotic testing.

6. Located on the side bench you will find Petri plates containing antibiotic disc cylinders. You will need penicillin, methicillin, tetracycline, ciprofloxacin, sulfadiazine and erythromycin. Write down the letters on the disks (or on your plate's under-surface, under the discs and agar) and the names of the corresponding antibiotics so you will be able to interpret your results next week. You will need at least one chrome disc-dispenser. Dispense three disks onto each plate, being careful to keep them as far apart as possible from each other, and from the edge of the plate (equidistant), so your zones of inhibition don't run together.

7. Sterilize a loop, and gently touch it to each disk to be sure the disc adheres to the surface of the medium. Don't push the disc into the media. Gently tap the disc and it will adhere.

8. Place your test plates in the green "save" bin. Discard your heat sink.

Throat Culture & Identification – SESSION 3

Antibiotic Sensitivity Testing Procedure

1. Retrieve the antibiotic sensitivity plates from the bin.
2. Identify antibiotics that lack a zone of inhibition, and record the result as "No zone of inhibition".
3. Identify antibiotic discs encircled by a zone of inhibition (Figures 6-1 & 6-2).
   a. Place the edge of the metric ruler on the back of the bottom of the TSY plate so that the zero hash mark is lined up with one edge of the zone.
   b. Measure the diameter of the zone in millimeters through the middle of the disc, and record the result in the second table that follows.
   c. Use the table on your Patient Work-up to interpret your results. S = sensitive, R = resistant, I = intermediate sensitivity

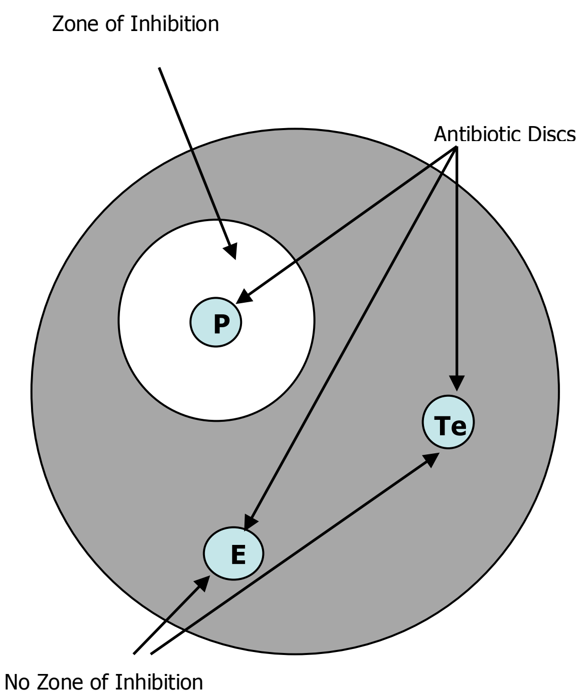

Figure 6-1: Antibiotic Discs on an Inoculated TSY Plate

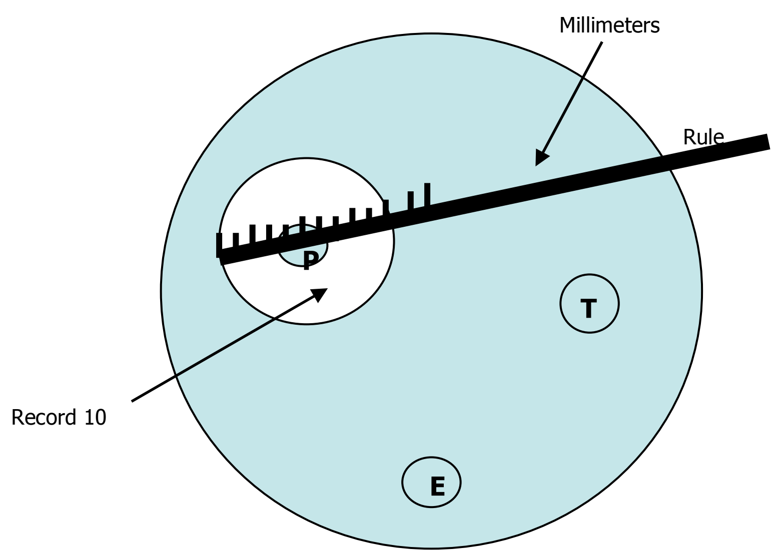

Figure 6-2: Measuring the Zone of Inhibition