

**Lab Exercise 2
Media and Culture**

I. OBJECTIVES:

- ✓ Practice microbial collection techniques
- ✓ Describe colony morphology and the relationship to microbial identification.
- ✓ Define and use aseptic technique in microbial culture and media preparation.
- ✓ Use selective, differential, enriched, and complex media to culture microbes.
- ✓ Analyze the results of microbial growth on various culture media.
- ✓ Use the terminology (listed) correctly.
- ✓ Understand the ubiquitous nature of microbes.

II. TERMINOLOGY: Students should define and use the following terms:

agar	culture	Media
alpha hemolysis	Enterobacteriaceae	normal flora
aseptic technique	Fermenter	selective media
autoclave	gamma hemolysis	<i>Staphylococcus</i>
beta hemolysis	Gram negative	sterile
blood agar	Gram positive	sterilization
Cell morphology	inoculum	Streak plate
Colony morphology	MacConkey's enriched media	<i>Streptococcus</i>
Complex growth media	mannitol salt	TSY

III. INTRODUCTION

Our bodies are composed of 100 trillion (10^{16}) cells. We are inhabited by a quadrillion (10^{17}) bacterial cells, known as **normal flora**. Normal flora is found on any exposed surface of the body, like skin and mucus membranes, but internal organs are sterile---no bacteria live there.

You are colonized with normal flora at birth. Most normal flora bacteria are long term inhabitants. Others, called transient flora, are just passing through: you may be colonized by these normal flora bacteria at various times, but not necessarily all the time. In this lab you will investigate the normal flora that resides on different surfaces of your body.

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 scant nutrients available (again, competing with potential pathogens) and put out waste products that keep other, less desirable, microbes from setting up shop.

Bacteria and other microbes have particular requirements for growth. When they reside in and on our bodies or in the environment, they harvest their food from us or from the environment. In order to successfully grow the bacteria in lab so that we can stain and identify them, we must provide an environment that is suitable for growth.

Growth media (singular: **medium**) are used to cultivate bacteria. Media are mixtures of nutrients that the microbes need to live. They also provide the necessary moisture and pH to support microbial growth. Bacterial cultures are incubated at temperatures known to be best for their growth. The bacteria that live on your body surfaces are mesophiles – they grow at temperatures between 25 and 45 °C

The medium that we use most often is designated "TSY" (**Tryptic Soy Agar**) and it is a complex nutrient medium which supports the growth of a wide variety of microbes. When the lab personnel make a medium, they measure out a designated quantity of dry powdered medium, add a designated amount of water and check the pH. They dispense the medium into bottles, cap it and autoclave it. Autoclaving is a process similar to home canning techniques of food preservation. Once the medium is **autoclaved** (or pressure cooked) it is considered sterile. The autoclave exposes the medium to high temperature (121°C) and pressure (15 psi) for 20 minutes. This exposure has been demonstrated to result in **sterilization** (Sterilization is the process of killing all life forms).

Many normal flora and clinically important microbes can be grown either in liquid medium (sometimes called **broth**), or on Petri dishes (also called Petri plates, or just "plates"). When grown on plates, **agar** is added to the liquid medium so that, when cool, the medium has the consistency of very stiff Jello®. Agar is an inert seaweed extract that solidifies at room temperature.

Other media used in the student lab is prepared and poured into Petri plates by the lab staff. The media provided already poured into plates and solidified includes MacConkey's, Blood agar and Mannitol Salt. MacConkey's and Mannitol Salt are described as selective and differential media; blood agar is an enriched differential media.

Next week you will examine the colonies that are growing on the agar as a result of the samples that you take this week. A colony looks like a dot growing on the medium and is composed of millions of bacteria that arose through binary fission from one initial bacterium. When you examine these colonies next week, you will be describing **colony morphology** (the appearance of the colony, including shape, color, surface features, etc.

Mac Conkey's Agar

MacConkey's is a **selective** media because it inhibits the growth of Gram positive bacteria due to the presence of crystal violet and bile salts. It is also **differential** meaning that it differentiates or distinguishes groups of bacteria on the basis of a color reaction based on whether or not the microbes growing on MacConkey's ferment lactose. MacConkey's contains two additives for this purpose: neutral red (a pH indicator) and lactose (a sugar). Bacteria, known as "lactose fermenters", metabolize the lactose to an acidic end product. This acidic metabolite causes the pH indicator, neutral red, to turn red. Thus the colonies of lactose fermenters will grow in pink/red colonies while non-lactose fermenters will be colorless (or more the color of the media prior to inoculation).

Members of the family Enterobacteriaceae (Gram negative bacilli typically found in the gastrointestinal tract of mammals) are the most frequently encountered bacteria isolated from many types of clinical specimens (e.g. urine). They are most commonly lactose fermenters.

Mannitol Salt Agar

Mannitol Salt is a **selective** medium because it has a high concentration of NaCl (7.5%). Most bacteria cannot survive in this environment. The genus *Staphylococcus*, however, grows well in this media. The medium is also **differential**: It contains a dye that identifies pathogenic *Staphylococcus* (*S. aureus*) as it produces an organic acid from mannitol fermentation and changes the pH indicator from red to yellow.

Blood Agar

Blood agar is an enriched medium meaning that it provides an extra rich nutrient environment for the microbes. Many specimens received in a clinical microbiology lab are plated onto blood agar, since it supports the growth of a wide range of organisms. Blood agar is a **differential** medium. Microbiologists can distinguish clinically significant bacteria in throat and sputum cultures easily on blood agar as a result of **hemolysis** patterns. Blood agar contains 5% sheep blood. Certain bacteria produce extra-cellular enzymes (**hemolysins**) that act on the red cells to lyse them completely (**Beta hemolysis**), to produce a greenish

discoloration around the colony indicating incomplete hemolysis (**alpha hemolysis**), or have no effect on the red cells (**gamma hemolysis**).

Blood agar is usually inoculated from a patient's throat swab. The microbiologist is trying to detect the presence of Group A beta hemolytic Streptococci (a Gram-positive cocci that causes 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.

Culturing Bacteria

In medical microbiology we routinely "**culture**" a particular site to investigate and find a microbe that would be the cause of a microbial disease. In this lab exercise you will culture various environments and body surfaces using media commonly found in the clinical laboratory for the purpose of investigating normal flora on the surface of our bodies and in the environment. We will do this by taking a sample and plating it out on nutrient media.

When a single cell comes in contact with the media it reproduces and its progeny reproduces and so on, until a mound of cells, called a colony, is visible. Visible colonies are composed of a million or more cells. Colonies of different bacteria and fungi have different characteristics. The **colony morphology** is often used to assist the microbiologist in identifying the microbe.

We will not be able to identify (genus and species identification) microbes from the colony morphology, but we will begin to understand that microbial world by understanding how widely microbes are distributed in nature. The microbes that you observe on the media plates are only the microbes that would grow under our laboratory conditions and may represent only a fraction of the microbes in the environment that you sampled.

IV. MATERIALS PER SET OF LAB PARTNERS (In addition to supplies found in your supply drawer):

- 6 Sterile Petri plates
- 1 "Arm" plate
- 1 bottle TSY
- 2 MacConkey's plates
- 1 Blood agar plate
- 1 Mannitol Salt plate
- Sterile cotton swabs in test tube
- 2 4-packs of sterile cotton swabs

V. PROCEDURE

A. Aseptically Pour Media

Aseptically pour 6 TSY plates and 1 "arm" plate using the following technique:

1. Obtain a bottle of TSY media from the warming oven. Careful, it is *warm/hot*. *You may need an insulated glove to protect your hand.*
2. Get the Bunsen burner out of your supply drawer, connect it to the gas supply, then arrange the six Petri dishes, and the one arm plate, so that they are near the Bunsen burner.
3. Carefully light the Bunsen burner on your table. (Care should be taken that there are no papers in the area and all long hair and loose clothing has been secured prior to ignition.)
4. Swirl the bottle of media to re-suspend the mixture.
5. Open the cap as demonstrated, flame the top of the bottle.
6. Open the Petri dish as demonstrated, pour about a ¼ inch of media into the plate.
7. Flame the bottle and return the cap.
8. Return the bottle to the warming oven. If the bottle is empty set it in the "discard" bin on the side bench in the lab.
9. Do not disturb the Petri dish until it has come to room temperature.

B. Obtain Environmental Sample (Get these samples from home.)

1. Obtain your plates. Each student will need a TSY and a MacConkey's plate.
2. Label your plates. (Identifying specimens in the clinical setting is very important. Observe that when writing on media plates, specimen collection cups or culturettes, you are directed to write on the portion of the plate that cannot be displaced. This same technique is used throughout the clinical setting. Writing on a lid that can be exchanged for a lid from a different culture plate would lead to error.) See Figure 1.

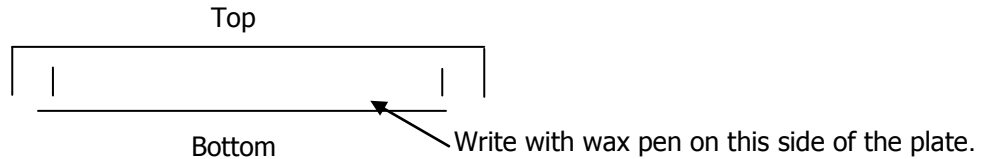


Figure 1: Plate Labeling

- a. Using a wax pen, divide the bottom (the part of the plate that contains the media) of the TSY plate into 4 pie shaped sections and label 1 through 4. (See Figure 2)
 - b. Divide the MacConkey's plate in the same manner.
 - c. Label each plate with your initials and the date.
 - d. Wrap a rubber band around the plate to hold it shut. (Normally we don't use rubber bands to hold plates shut. This is only for the plates that you are bringing home.)
 - e. Each student will take a 4-pack of sterile swabs to collect your samples. When obtaining each environmental sample, for each sample streak the TSY first and the selective MacConkey's second.
3. You will be bringing these plates home and taking environmental samples from your bathroom and kitchen. Use a new, sterile swab for each location that you are sampling. Remember, for each sample streak the TSY first and the selective MacConkey's second.
 - a. Quadrant 1: Obtain a sample from your kitchen counter.
 - b. Quadrant 2: Obtain a sample from your kitchen sink.
 - c. Quadrant 3: Obtain a sample from your bathroom counter.
 - d. Quadrant 4: Obtain a sample from your bathroom sink.
 4. After obtaining the samples, close the plate, place the rubber band around it to hold it shut, and put the plate on top of your refrigerator, upside down (media-side up). Remember to bring this plate back to school for our next lab!

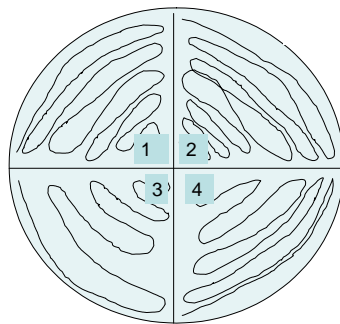


Figure 2: Streak Patterns for Environmental Specimens

C. Obtain Samples of Normal Flora

1. "Arm Plate" will demonstrate the microorganism inhabiting the surface of the skin.
 - a. Remove the small cup from the Petri dish after the agar has solidified.
 - b. Tape the cup to your forearm so that the agar is in contact with your skin.
 - c. After 10 minutes, remove the cup from your arm and return it to the Petri dish.
 - d. Incubate the Petri dish until next week. (NOTE: This is the ONLY plate that will be incubated 'media side up'. You will need to label the top/lid of the glass Petri plate).
2. Touch plates will demonstrate the bacteria in the tips of your fingers.
 - a. Obtain 3 TSY plates, and number them Touch 1, Touch 2, and Touch 3.
 - c. Remove the lid from plate 1 and touch lightly with fingers as demonstrated.
 - d. Wash your hands with soap and touch plate #2.
 - e. Scrub the same fingers with a brush and hand soap then touch plate # 3. (NOTE: rinse the alcohol off the brush first.)
 - f. Store the plates.
3. Throat swabs will demonstrate normal oral flora.
 - a. Obtain a Blood Agar Plate.
 - b. Using the wax pen, draw a line down the middle of the plate dividing it into two halves (Draw the line on the bottom plate, where you label.)
 - c. Use a sterile swab to take a sample from the back of your throat.
 - d. Plate out the swab on one half of the Blood Agar Plate. Your lab partner will plate his/her sample on the other half of the plate. Make sure to apply your sample to the half of the plate that you have labeled.
4. Nasal swabs will demonstrate normal nasal flora.
 - a. Obtain a sterile cotton swab, and TSY and Mannitol Salt Agar plates.
 - b. Using the wax pen, draw a line down the middle of the Petri plate dividing the plate into two halves (draw the line on the media side of the Petri plate).
 - c. Gently swab the inside of your nose.
 - d. Plate out the swab on one half of the TSY and Mannitol Salt media plates. Swab the TSY plate first and then, **without** re-culturing your nose, use the same cotton swab to inoculate the Mannitol Salt plate. Make sure to apply your samples to the halves of the plates that you have labeled.

D. Label and Store

All Petri plates used in lab exercises should be labeled and stored in the following manner:

1. Make certain that all plates are labeled on the bottom half (i.e. the portion of the Petri plate that contains the media).
2. Label with a wax pen.
3. Include the following:
 - a. Your initials or identifying mark
 - b. Date
 - c. Type of specimen
4. Always store plates in the green 'Save' bin as follows: Labeled with your initials and the date on the media side of the plate; Stored upside down (or media side up); No tape or rubber bands; Write down the total number of plates you have stored so you can retrieve your samples the following week with certainty.
5. Plates will be incubated at 37° C for 24 hours and then removed and stored at room temperature (or refrigerated if necessary) until next week when you will observe for results.

**Always wash down your bench
with disinfectant at the end of the lab time.**