**Lab Exercise 4 - INSTRUCTIONS**

**Identification of Unknown Bacteria**

**Bacterial Growth Media and Culture**

* Aseptically pour TSY agar
* Set up bacterial controls on TSY, Mannitol Salt, MacConkey’s & Blood agar
* Evaluate your bacterial unknown from isolation streak plates created last week on MAC & MSA
* Obtain normal flora samples

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 | *Staphylococcus* |
| beta hemolysis | Gram negative | sterile |
| blood agar | Gram positive | sterilization |
| Cell morphology | inoculum | Streak plate |
| Colony morphology | MacConkey's enriched media | *Streptococcus* |
| Complex growth media | mannitol salt | TSY |

III. INTRODUCTION

Our bodies are composed of 100 trillion (1016) cells. We are inhabited by a quadrillion (1017) 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. These sites are said to be axenic.

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 oC

You have had some experience using TSY agar in lab #2. Additionally, you set up isolation streak plates of your unknown on MacConkey’s and mannitol salt agar in lab #3 which we will investigate today. We will use these media again and introduce blood agar as a tool for microbial identification.

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**

See the Lab #3 instructions for details regarding MacConkey’s media.

**Mannitol Salt Agar**

Refer to Lab #3 instructions kfor information about Mannitol Salt agar.

**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)**:**

5 Sterile Petri plates

1 “Arm” plate

1 bottle TSY

1 MacConkey’s plates

2 Blood agar plate

2 Mannitol Salt plate

Sterile cotton swabs in test tube

### V. PROCEDURE

## A. Aseptically Pour Media

Aseptically pour 5 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. Inoculate Plates with Controls

1. Each pair of lab partners will do one set of controls as a team: 1 TSY, 1 MacConkey’s , 1 Mannitol Salt, 1 Blood Agar plate.

2. Label your plates using the technique you learned in Lab #2.

3. Using a wax pen, divide the bottom (the part of the plate that contains the media) of the TSY plate into 5 sectors shaped sections and label 1 through 5. (See Figure 1)

b. Divide the MacConkey’s, Mannitol Salt, and Blood Agar plates in the same manner.

c. Label each plate with your initials and the date.

4. Inoculate plate sectors with the following bacteria:

* 1. Sector 1: *Staphylococcus aureus*
  2. Sector 2: *Staphylococcus epidermidis*
  3. Sector 3: *Escherichia coli*
  4. Sector 4: *Salmonella pullorum*
  5. Sector 5: Sterilize your inoculating loop and do not pick up any bacteria. Drag the sterile inoculating loop over the media in the same manner in which

you inoculated the bacteria cultures. This is your negative control.

1

5

2

4

3

Figure 1: Inoculation pattern for control plate

## 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. You 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.

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### 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.

**D. Read the Results**

The following week the incubated plates will be available in the laboratory so you can see the results of the bacterial inoculations.

1. Recall the number of plates you must analyze and be certain to carefully pick up all your plates from the storage bin. Care must be taken that the lids are not displaced. Also, if moisture is trapped in the plate you must be VERY CAREFUL that it does not leak out of the plate and contaminate you. DO NOT juggle plates or use your body to stabilize a large stack of plates you are trying to carry. Rather, enlist the aid of your lab partner and carefully transport 3 or 4 plates held in a horizontal position away from your body.
2. Once back at your bench organize the plates, media side up, on your bench. Do not remove the lids.
3. Observe your control plate.

### Always wash down your bench

**with disinfectant at the end of the lab time**.

This material is adapted from the Applied Microbiology Laboratory Manual by Cynthia Schauer. For Power Point slides that correspond to this lab material, see the Virtual Microbiology Classroom of the [Science Prof Online](http://www.scienceprofonline.com) website.