SDM1 Selective and Differential Media 1

Learning Objectives

The student will

- Use aseptic techniques in the safe inoculation of various forms of media.
- Follow oral and written instructions and manage time in the lab efficiently.
- Apply correct terminology regarding microbiological techniques, instruments, microbial growth, biochemical testing, and media types when making observations.
- Correctly perform various inoculation techniques and describe each technique's purpose.
- Make accurate observations and appropriate interpretations of biochemical test results and use them in the identification of potentially disease causing microbes.

Background/Theory

In this course you will encounter many types of growth media. Most of the types, TSA/TSB (Tryptic Soy Agar/Broth) and NA/NB (Nutrient Agar/Broth) for example, are **all purpose or general media**. They contain a wide variety of complex carbon, nitrogen and sugar compounds. You will often see terms like beef extract, peptone, tryptone, soytone in the list of ingredients. These components are slurries of animal and plant tissue that have been partially hydrolyzed (broken down) so that the components are more readily available. These media contain many different complex molecules, however the exact amount and types of each is unknown. These media will support a wide range of **nonfastidious** microbes with differing nutritional requirements.

Media that inhibit the growth of unwanted microorganisms and support the growth of the organism of interest by supplying nutrients and reducing competition are called **selective media**. (OpenStax CNX, 2018) Selective media are formulated with inhibitors such as antibiotics or high NaCl concentration. When studying a mixed sample, selective media can be helpful. For example, if you suspect a patient is carrying *Salmonella* (a pathogenic Gram negative bacillus), you may plate a stool sample on a selective medium containing an antibiotic effective against Gram positive bacteria. By eliminating the Gram positive organisms, the range of organisms growing on the plate will be narrowed to Gram negatives. Thus, the variety of bacteria you will need to study is reduced.

The fact that a medium does not grow every microbe, does not make it selective. For example, TSA is an all-purpose medium and a wide range of organisms grow on it. Certain fastidious organisms, however, will fail to grow or grow poorly on TSA because it lacks the specific nutrients required by those bacteria. Even so, TSA is not classified as selective. To be selective, a medium must contain a specific substance intentionally added to inhibit certain microbes and not others.

Differential media contain substrates and indicators (often pH indicators) that make a certain biochemical process visible. Differential media allow one to differentiate between types of organisms growing on the plate because each has a distinct appearance based on whether or not it is carrying out a particular biochemical reaction. "Color changes are the result of end products created by interaction of bacterial enzymes with differential substrates in the medium or, in the case of hemolytic reactions, the lysis of red blood cells in the medium" (OpenStax CNX, 2018). Differential media can be used to distinguish between bacteria that can ferment a specific type of sugar and those that cannot or between bacteria that utilize a certain electron acceptor and those that do not.

Some media are selective, some are differential and some are both. We will study Mannitol Salt Agar and MacConkey agar as examples.

Mannitol Salt Agar (MSA) can be used to presumptively isolate and identify *Staphylococci* from human samples. Refer to the compositions of MSA and MacConkey agar below. MSA contains 75 g/L NaCl (7.5%) compared to the 5 g/L found in TSA and other all-purpose media. MSA favors the growth of salt tolerant microbes, namely *Staphylococci*, because other bacteria from a human sample, are inhibited by the high NaCl component. In addition, to distinguish pathogenic *Staphylococci, namely S. aureus* from other common *Staphylococci,* the substrate mannitol (a sugar) and the pH indicator phenol red are added. If the organism ferments mannitol, acids will be produced as byproducts. These acids will lower the pH changing the indicator from pink to yellow. *S. aureus* can ferment mannitol, while other common *Staphylococci* found in humans cannot.

MacConkey agar contains bile salts and crystal violet, which interfere with the growth of many gram-positive bacteria and favor the growth of gram-negative bacteria, particularly the **Enterobacteriaceae**. These species, commonly named **enterics,** reside in the intestine, and are adapted to the presence of bile salts. Enterics can be further characterized by their ability to ferment lactose. In MacConkey agar, the lactose fermenters (**coliforms**) utilize lactose in the medium producing acid, lowering the pH. The medium is supplemented with the pH indicator neutral red, which turns to hot pink at low pH. (OpenStax CNX, 2018) Thus, lactose fermenters are observed as bright pink colonies or with a bright pink halo surrounding the growth. Non-lactose fermenters (noncoliforms) include some notable human pathogens, such as *Salmonella* spp., *Shigella* spp., and *Yersinia pestis*. (OpenStax CNX, 2018)

Coliform bacteria are microbes found in the digestive systems of warm-blooded animals, in soil, on plants, and in surface water. (Note their ability to assist mammals in the digestion of milk sugar, lactose.) These microbes typically do not make you sick; however, because microbes that do cause disease are hard to test for in the water, “total coliforms” are tested instead. If the total coliform count is high, then it is very possible that harmful germs like viruses, bacteria, and parasites might also be found in the water. Thus, they are considered one of several a water quality indicators. (U.S. Centers for Disease Control and Prevention, 2019)

MacConkey Agar (MacC)

Pancreatic digest of Gelatin 17g/L
Peptones (meat and Casein) 3g/L
Lactose 10g/L
Bile Salts 1.5g/L
Sodium Chloride 5g/L
Agar 13.5g/L
Neutral red 0.03g/L
Crystal Violet 1mg/L

Mannitol Salts Agar (MSA)

Pancreatic digest of Casein 5g/L
Peptic digest of Animal Tissue 5g/L
Beef extract 1g/L
Sodium Chloride 75g/L
D-Mannitol 10g/L
Phenol red 25mg/L
Agar 15g/L

Tryptic Soy Agar (TSA)

Tryptone 17g/L
Soytone 3g/L

Sodium Chloride 5g/L
Dipotassium Phosphate 2.5g/L
Agar 15g/L

Experiment/Exercise

Materials per student pair

1 TSA plate
1 MSA plate*
1 MacConkey Agar plate*
* Be sure to label each as you take it. They look very similar!

Cultures

Fresh overnight broth cultures
E. coli
Staphylococcus epidermidis
Staphylococcus aureus (Risk Group 2, BSL-2 precautions)
Enterococcus faecalis
Pseudomonas fluorescens

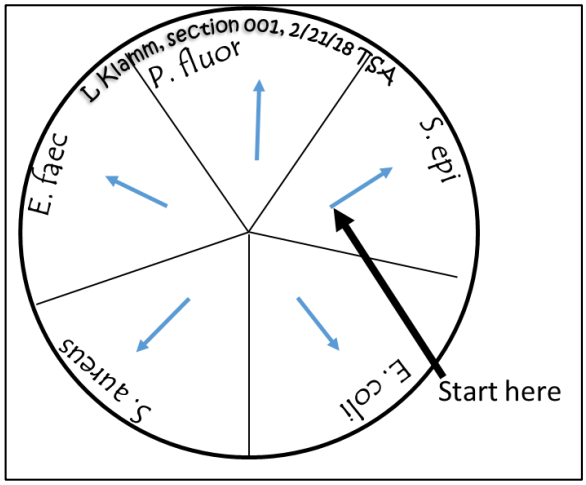

Figure 1 Spot Inoculation of a TSA plate.
Inoculations should be made toward the plate's edge, like spokes of a wheel. Keep the line short!

Procedure Lab 1

1. Notice that you are using an organism from Risk Group 2 this week! Use extra caution.
2. Obtain one TSA plate, one MSA plate and one MacConkey agar plate. On the plate base write the medium abbreviation when you remove it from the bag on the media cart.
3. Add the following to the bottom of each plate around the edge: your name, section, date.
4. Divide each plate into 5 sections.
5. On the bottom, divide each plate into 5 sections. Label each section with an abbreviation for each organism. Write small but legibly! Each plate will be inoculated with each of the 5 organisms.
6. Aseptically spot inoculate each sector with the corresponding microbe. A **spot inoculation** is a short (1 cm) streak line as shown in figure 1. (DO NOT STAB the agar.)
   - Keep the inoculation lines short and away from the other inoculations on the plate.
   - Be sure to make each inoculation separately and refrain from "double dipping."
   - Be sure to hold the lid of the plate above the plate surface to protect it from airborne contaminants.
   - It may help to set the plate on a piece of white scratch paper so that you can see the sector lines.
7. Place the plates upside-down in the location designated for cultures to be incubated.
8. They will be incubated for 24-48 hours at 37°C.
9. After other students are finished with the parent cultures, dispose of them on the disposal cart.

Procedure Lab 2

1. Obtain your plates and make observations in the data table.
2. Use + for growth and – for no growth. If growth is poor, simply write "poor growth" in the table.

3. In the appearance column, describe any color change in the growth and/or the surrounding medium. If the organism did not grow, you will not be able to describe an appearance. In this case write "N/A" in the table.
4. Be sure to use the organism's full scientific name written correctly.
5. After making observations, dispose of your plates in the container on the disposal cart.

Lab Report: Selective and Differential Media 1

Name _____
Lab Section _____

Data and Observations

Organism	Growth on TSA +/-	Appearance on TSA	Growth on MacC +/-	Appearance on MacC	Growth on MSA +/-	Appearance on MSA

Post Lab Questions

1. What is the purpose of the TSA plate? What specific information does it provide?

2. Based on your observations in this exercise, you should be able to list several characteristics of each organism. Fill in this interpretation table. If you cannot make a determination based on these results, write in "N/A." (Since you have already written the full scientific name of each organism, you may appropriately abbreviate them here.)

   Interpretation Table

Organism	Gram positive/Gram negative	ability to ferment lactose	salt tolerance	ability to ferment mannitol

3. Compare the interpretation table above to your results in SDM GSt. Are there any discrepancies? If so list them and give a brief reasonable explanation.

4. Is MSA selective, differential or both? What ingredient(s) make it so?

☐ Selective: Ingredients: _____

☐ Differential: Ingredients: _____

5. Is MacC selective differential or both? What ingredient(s) make it so? (Use the format in #5.)

6. EMB agar is a medium used in the identification and isolation of pathogenic bacteria. It contains digested meat proteins as a source of organic nutrients. Two indicator dyes, eosin and methylene blue, inhibit the growth of gram-positive bacteria and distinguish between lactose fermenting and non-lactose fermenting organisms. Lactose fermenters form metallic green or deep purple colonies, whereas the non-lactose fermenters form completely colorless colonies. EMB agar is an example of which of the following?
   a. a selective medium only
   b. a differential medium only
   c. a selective medium and a chemically defined medium
   d. a selective medium, a differential medium, and an undefined/complex medium

   (OpenStax CNX, 2018)

7. A patient presents with an oozing, pus-filled skin lesion. You suspect a *Staphylococcus aureus* infection. What medium would you use to plate a sample from the lesion? What result do you expect if the *Staphylococcus aureus* is present?

References

OpenStax CNX. (2018, Mar 19). OpenStax Microbiology. Retrieved from http://cnx.org/contents/e42bd376-624b-4c0f-972f-e0c57998e765@4.24

U.S. Centers for Disease Control and Prevention. (2019, June 30). *Well Testing*. Retrieved from Healthy Water: https://www.cdc.gov/healthywater/drinking/private/wells/testing.html