SDM2 Selective and Differential Media 2

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

Hemolysis, the ability of an organism to lyse red blood cells and breakdown hemoglobin can be seen when sheep's blood is added to the medium. Substances produced and released (exotoxins) which accomplish this are called **hemolysins**. The addition of blood has a two-fold purpose. It is an **enrichment** to support the growth of fastidious organisms and it is the substrate with which hemolysins, if present, will interact. In blood agar, the hemolytic activity can be visualized without an indicator substance usually added to most differential media.

Before inoculation, a **blood agar** plate appears red and opaque due to the large particulate red blood cells (RBCs) contained within. If an organism growing on the plate produces **beta (β) hemolysins**, the RBCs are completely destroyed or lysed and the medium surrounding the growth loses its opacity. If this is the case, the medium may become transparent enough that you may be able to read printing through the clearing.

If the blood cell membranes are partially lysed by the exotoxin, the RBC contents will leak out without the complete destruction of the blood cell. The hemoglobin from the RBC will be reduced to methemoglobin resulting in a green or brown discoloration to the medium surrounding the colony (Buxton, 2005). This incomplete hemolysis is called **alpha (α) hemolysis. "**On prolonged incubation, many alpha hemolytic organisms will begin to appear more clear, but if the surrounding medium contains any shades of brown or green the 'hemolysis' is still considered 'alpha.'" (Buxton, 2005)

If the bacterium does not produce a hemolytic exotoxin, there will be no change to the RBCs, and the medium will remain opaque red. This lack of hemolysis is classified as **gamma (ϒ) hemolysis**.

Adding a couple of antibiotics and some extra enrichment ingredients to blood agar, will produce **Columbia CNA**. The antibiotics, colistin and nalidixic acid (CNA) inhibit the growth of Gram-negative organisms making Columbia CNA selective as well as differential. Colistin affects cell membrane formation and nalidixic acid interferes with DNA replication.

Hemolysins are involved in the pathogenicity of bacteria. These membrane-disrupting **exotoxins** affect cell membrane function either by forming pores or by disrupting the phospholipid bilayer in host red blood cell membranes. They cause leakage of the cytoplasmic contents and cell lysis. They can affect other cells as well. The gram-positive bacterium *Streptococcus pyogenes* produces two types of streptolysins, O and S. Streptomycin O is not active in the presence of oxygen (**oxygen labile**), whereas streptolysin S is active in the presence of oxygen (**oxygen stable**). Other important pore-forming membrane-disrupting toxins include alpha toxin of *Staphylococcus aureus* and pneumolysin of *Streptococcus pneumoniae*. (OpenStax CNX, 2018)

Blood Agar (TSB w/ 5%SB)
Pancreatic digest of casein 14.5 g/L
Peptic digest of soybean meal 5.0 g/L
Sodium chloride 5.0 g/L
Agar 14.0 g/L
Defibrinated Sheep Blood 5.0%

Columbia CNA w/ 5% SB (CNA)
Pancreatic digest of casein 12.0 g/L
Peptic digest of animal tissue 5.0 g/L
Yeast extract 3.0 g/L
Beef extract 3.0 g/L
Corn starch 1.0 g/L
Sodium chloride 5.0 g/L
Nalidixic acid 10.0 mg/L
Colistin 10.0 mg/L
Agar 13.5 g/L
Defibrinated Sheep Blood 5.0%

Experiment/Exercise

Materials per student pair
1 blood agar plate (TSA w/ 5% SB)
1 Columbia CNA plate

Cultures
Fresh overnight broth cultures
Pseudomonas aeruginosa
Streptococcus pyogenes (Risk Group 2, BSL-2 precautions)
Enterococcus faecalis
Streptococcus pneumoniae (Risk Group 2, BSL-2 precautions)

Procedure Lab 1
1. Notice that you are using organisms from Risk Group 2 this week! Use extra caution.
2. Obtain one blood agar plate and one CNA plate from the media cart. Make sure that you have one of each….they look identical.
3. Add the following to the bottom of the plate around the edge: your name, section, date. The media type is already stamped on the plate.
4. On the bottom, divide each plate into 4 sections. Label each section with an abbreviation for each organism. Write small but legibly! Each plate will be inoculated with each of the 4 organisms. (These are not the same organisms you used in SDM1!)

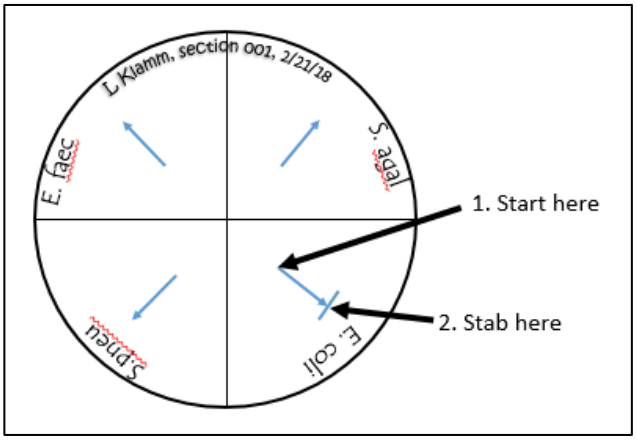

Figure 1 Streak Stab Inoculation of a Blood Agar Plate
Streak on the surface toward the plate's edge. Stab the surface at the end of the streak. Keep the lines short.

5. Aseptically make a short (1 cm) streak-stab inoculation of each organism in the corresponding section. Refer to figure 1. After obtaining cells on the loop, begin the surface streak at point 1 and ending with a stab at point 2. Inoculate each organism into its area with a short straight line/spot inoculation followed by a stab at the end of the streak line.
   - Keep the inoculation lines short and away from 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.
6. Place the plates upside-down in the location designated for cultures to be incubated.
7. They will be incubated for 24-48 hours at 37^{o}C.
8. 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 change in the surrounding medium or the growth itself. Hold the plate up to the light. You may see slight lighting of the medium or a complete clearing. The medium or the growth itself may appear green. Careful not to confuse a greenish appearance with a shadow created by the growth. If there is no change in appearance to the medium, write "no change."
4. Be sure to use the organism's full scientific name written correctly.
5. After making observations, dispose of plates in the container on the disposal cart.

SDM2

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Lab Report: Selective and Differential Media 2

Name _____
Lab Section _____

Data and Observations

Organism	Growth on Blood agar +/-	Appearance of surface streak on blood agar	Appearance of the stab on blood agar	Growth on CNA +/-	Appearance of surface streak on CNA	Appearance of the stab on CNA

Post Lab Questions

1. Is blood agar selective, differential or both? What ingredient(s) make it so? (Use the format in SDM1 Post Lab question 5.)

2. Is CNA selective, differential or both? What ingredient(s) make it so? (Use the format in SDM1 Post Lab question 5.)

3. What is the purpose of stabbing the agar? What information will it provide about the hemolysins produced by the organism?

4. Based on your observations <u>in this exercise</u>, you should be able to list some characteristics of each organism. Fill in this interpretation table. (Since you have already written the full scientific name of each organism, you may appropriately abbreviate them here.)

Interpretation Table

Organism	Gram positive/negative	(Oxygen stable) Hemolysis type	(Oxygen labile) Hemolysis type

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

6. Do your results correlate with the pathogenicity of the BSL-2 organisms used in this exercise? Explain <u>concisely</u>.

7. You suspect a patient is suffering from strep throat. You swab the back of their throat with a sterile cotton swab. What type of medium is the best choice for your zig zag inoculation? What would you be looking for after incubation that would support your diagnosis?

References

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