DTSIM SIM Medium

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, media types and forms 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

SIM is an example of a **combination medium**, meaning that one can determine several bacterial activities/characteristics through the use of one medium. SIM medium tests for **s**ulfur reduction, **i**ndole production and **m**otility. The form of medium used for this test is an **agar deep**. SIM Medium contains the following: pancreatic digest of casein, peptic digest of animal tissue, ferrous ammonium sulfate $Fe(NH_4)_2(SO_4)$, sodium thiosulfate $Na_2S_2O_3$, agar (3.5 g/L) and distilled water.

Sulfur can be reduced producing hydrogen sulfide by bacteria in two unrelated ways. One process occurs during putrefaction. When proteins putrefy, the resulting foul "rotten egg" smell is due to the production of hydrogen sulfide gas, H_2S. Hydrogen sulfide is a byproduct of the conversion of the amino acid cysteine to pyruvate by the enzyme **cysteine desulfurase**. The second mode of H_2S generation involves anaerobic respiration. In some prokaryotes, thiosulfate ($S_2O_3^{2-}$) is the terminal electron acceptor in an anaerobic ETS. When thiosulfate is reduced (picks up electrons) the result is H_2S gas. In either case, invisible H_2S gas is produced.

Because hydrogen sulfide gas is colorless (though not odorless!) SIM medium uses an indicator reaction. Iron (supplied by ferrous ammonium sulfate) in the medium combines with H_2S gas to form iron sulfide, FeS, a black precipitate. Any black color in the medium is a positive test for sulfur reduction. Unfortunately, this test does not distinguish between the hydrogen sulfide produced as a result of putrefaction and hydrogen sulfide produced at the end of an anaerobic ETS.

Indole is produced during the conversion of tryptophan, an amino acid, to pyruvate and ammonia by the enzyme **tryptophanase**. Indole production indicates tryptophanase activity. Kovac's reagent, added after incubation, will turn pink when it combines with indole.

Motility is the ability of a microbe to "swim" using flagella. The reduced agar content of this medium, 3.5 g/L compared to 12-15 g/L in most solid media, creates a semi liquid environment allowing motile cells to spread from their original placement. The **stab technique** (see the description below) deposits cells in a straight line down the center of the deep. If growth is observed beyond the stab line into the periphery of the tube, the test is positive for motility. Avoid confusing growth produced by the lateral movement of the needle during an imperfect stab inoculation with actual motility. Rotating the tube for a side view and comparing each experimental tube to the sham inoculated tube will help you determine if growth is confined to the original inoculation line, or has truly spread into the periphery of the tube.

Stab Inoculation

1. Aseptically obtain cells on the end of the inoculating needle.
2. Holding the deep tube in your non-dominant hand, remove the cap and flame the mouth of the tube as you normally do with a tube.
3. Holding the needle vertically, stab the agar straight down the center to within a quarter inch of the bottom. Then draw it straight back out of the tube. Try to follow the original stab line when removing the needle. See figure 1.
4. Again, heat the mouth of the tube after withdrawing the transfer instrument. Replace the cap and set the tube in the test tube rack.
5. Immediately incinerate the inoculating needle for a full 10 seconds before setting it down.

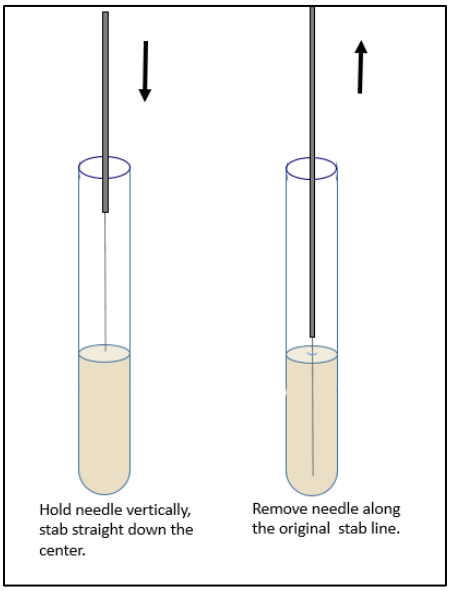

Figure 1 Stab inoculation

Materials per student pair

3-4 SIM deep tubes
Inoculating needle
Lab 2: Indole reagent (Kovac's) dropper tube (share with another pair)

Cultures

Fresh overnight plate cultures
Serratia marcescens
Citrobacter freundii (Risk Group 2, BSL-2)
E. coli

Procedure Lab 1

1. You are using a BSL-2 organism. Take care.
2. Label three of the deeps with each of the organisms. Don't forget to include the other components of the label.
3. For the forth deep, label it "sham inoculation." This tube may be done for the entire class by the TA.
4. First, complete the sham inoculation tube. After sterilizing your needle, aseptically perform a stab inoculation without cells. Even though this process should be sterile, be sure to incinerate the needle before the next transfer.
5. Aseptically obtain an inoculum from a plate culture. It should be substantial but does not need to be particularly heavy. Aseptically stab inoculate the corresponding tube.
6. Aseptically inoculate the other tubes in the same way.
7. Place the tubes for incubation for 24-48 hours at 37°C.

Procedure Lab 2

1. Make observations for sulfur reduction and motility first. As in the other DT exercises, observations are what you see, result is "+" or "-"and interpretation refers to the result's meaning.
2. In the sulfur reduction data table, observe the location of any black color.
3. For motility, it helps to compare the experimental tubes with the sham inoculated tube. Hold a paper with small print behind the tubes. Try to read the printing through the tubes. By comparing

the inoculated tubes with the sham, you can determine if there is radiating (fuzzy) growth from the stab line. Be sure that you can distinguish between non-motile growth confided to the stab line (in 2 dimensions) and actual radiating growth, 360^{o} around the inoculation.

4. After you have observed motility and sulfur reduction, you can add the reagent for the indole test. The Kovac's reagent is contained in small dropper tubes labeled "Indole." To open the indole dropper, crush the inner ampule by squeezing the tube between your thumb and forefinger.
5. Place 3-4 drops of the reagent on the agar surface. Replace the test tube cap. If indole is present, you will see a pink color develop within 2-3 minutes. Share the open dropper with another group.
6. Do not forget to observe the sham inoculated tube.
7. When finished, place your experimental tubes in the racks of the tube disposal tub. After sharing the indole reagent dropper with another group, dispose of it in the chemical disposal container labeled "Indole reagent" or "Kovac's Reagent" in the chemical fume hood.

DTSIM

Blank

Lab Report: SIM Medium

Name _____
Lab Section _____

Data and Observations

Sulfur Reduction

Organism (write name out fully)	Observations (include color and location)	Result +/-	Interpretation
Sham control			

Motility

Organism (may properly abbreviate)	Observations	Result +/-	Interpretation
Sham control			

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Indole Production

Organism (write name out fully)	Observations (include color and location)	Result +/-	Interpretation
Sham control			

Post Lab Questions

1. Would this medium be considered selective, differential or both?

2. What is the purpose of the sham inoculated tube?

3. The inability of this test to distinguish between H_2S produced by putrefaction and H_2S produced by reduction in the final step of an anaerobic ETS, can be described as poor _____ in this test system.

4. The indole test determines if the organism has the enzyme tryptophanase.
   a. The substrate for this enzyme is _____.
   b. What component of the medium supplies this substrate?

5. Predict what your observations <u>would look like</u> for *Citrobacter freundii* if you made each of these mistakes in preparing the medium. Explain in one sentence.
   a. Used 15 g/L agar

   b. Left out the ferrous ammonium sulfate $Fe(NH_4)_2(SO_4)$

c. Left out sodium thiosulfate $Na_2S_2O_3$

5. If you left out the sodium thiosulfate, as in 5c above, would that increase the specificity or sensitivity of the test?

References

There are no sources in the current document.