

## Lab #2: The Need for Sterile Technique

**Summary:** As a follow up to the lab in which students learn sterile technique, this lab allows students to actually use their Petri dishes in an actual experiment while reinforcing the concepts introduced in the previous lab.

**Grade Levels:** 9-12

**Prior Knowledge:** Students should be familiar with sterile technique as described in the previous lab. Also, students should know how to develop a hypothesis and explain why they have made the hypothesis they made. Students should know what to look for to describe bacterial colony morphologies.

**Materials:** In all labs in this series, the agar tryptic soy agar is used. This is only one of several media that are suitable. Others, such as Nutrient Agar or LB agar will also work. All students should practice the skills found in this lab, though material can be shared between pairs or groups of three students. More than three to a group may mean too much down time for students and the lab will not be finished in a reasonable amount of time. All of the following quantities are for pairs of students, unless otherwise noted.

60 plates of TSA (2 Petri dishes per student)

15 test tubes sterile TSA for pouring (enough for two plates in each test tube)

30 unsterilized Petri dishes

30 tubes of sterile nutrient broth

15 test tube brushes

30 5ml sterile pipettes

15 fine-tipped marking pen

tape (one roll /lab station)

30 sterile and empty test tubes

biohazard bag, parafilm and scissors (one set for the class)

### Teacher Instructions:

- I have actually had problems getting contamination using some of the techniques described in this lab. This may especially be an issue in newer buildings with filtered air systems. You may want to take measures to ensure that students actually see contamination when they are supposed to so they do not become too casual in their technique.
- For Part 2 of this lab, you will have to supply the students with diagrams or photographs of examples of various colony morphologies. You can search the Internet by using the phrase “colony morphology” on your favorite search engine. Several sources that you might try include the following:

[www.sci.tamucc.edu/pals/maric/Index/WEBPAGE/Dlab6.html](http://www.sci.tamucc.edu/pals/maric/Index/WEBPAGE/Dlab6.html)  
[www.slic2.wsu.edu:82/hurlbert/micro101/pages/101lab4.html](http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/101lab4.html)  
[www.austin.cc.tx.us/microbugz/labindex.html](http://www.austin.cc.tx.us/microbugz/labindex.html)

- Keep the agar for pouring liquid by placing in a water bath at about 50°C.
- If you use cotton for stoppering the broth, be sure to use only non-absorbent cotton.
- The sterile empty test tube can be prepared by covering the mouth of a test tube with a small piece of foil then autoclaving.
- The unsterile Petri dishes should be well used and scratched up for best results.
- You may wish to have students write a hypothesis only for Part 2, step 2 of this lab.
- In steps one and two of the Results, emphasize that #1 is looking at gross population, while #2 is looking at the diversity of organisms.
- Always have students dispose of their Petri dishes in the biohazard bag.
- If you wish to make LB agar, use the following recipe for 1 liter of agar (makes enough for about 90 60x15mm Petri dishes:

5g yeast extract (Fisher, #DF0127-15-1, 100g, \$32.05)

10g Bacto-Tryptone (Fisher, #DF0123-15-5, \$22.55)

5g NaCl

15g agar (Fisher, #DF0140-15-4, 100g, 61.95)

H<sub>2</sub>O to 1L

Mix the ingredients. To ensure complete dissolution, place the flask containing LB agar in a pan of water and boil the water, swirling the contents of the flask occasionally, until there are no solid particles seen in the solution. Pour the LB agar into two 500ml flasks and stopper the flasks with nonabsorbent cotton. Autoclave the agar at 121°C, 20 psi for 30 minutes. If you do not have an autoclave, you can pressure-cook it for 1 hour. Allow the media (LB agar) to cool to 55°C, then pour the plates using the sterile technique described in this lab.

Allow the plates to cool on the bench-top for as long as possible (at least overnight, but being out for 2-3 days will help to eliminate condensation forming on the lids). To check for contamination, you may wish to place an uninoculated dish in an incubator at 37°C overnight. No growth of bacteria means no contamination. Store the dishes in the sleeves they came in, upside down in the refrigerator. Do not store plates longer than several weeks.

- To make LB broth to replace nutrient broth, use the above recipe, except do not add the agar. Broth can be sterilized in stoppered, half-filled test tubes. Store the test tubes in the refrigerator until used, but do not store them for more than several weeks.

### **Correlations to State and National Standards:**

- **Colorado State Standard 3:** Life Science-- Students know and understand the characteristics and structure of living things, the processes of life, and how living things interact with each other and their environment.

- **Colorado State Standard 5:** Life Science-- Students know and understand interrelationships among science, technology, and human activity and how they can affect the world.
- **Colorado State Standard 6:** Life Science--Students understand that science involves a particular way of knowing and understand common connections among scientific disciplines.
- **National Content Standard C (Life Science):** As a result of their activities in grades 9-12, all students should develop understanding of the cell; the molecular basis of heredity; biological evolution; interdependence of organisms, matter, energy, and organization in living systems; and behavior of organisms.

**Correlation to Confronting the Microbe Menace:**

**Supplementary Materials:**

**Materials Price List/Ordering Information:**

Carolina 1-800-334-5551 [www.carolina.com](http://www.carolina.com)

Item	Ordering Number	Price
Bacterial Spreader	BA-21-5820	\$2.70 each
Inoculation Loop	BA-21-5826	\$1.80 each
TSA plates (100 x 15 mm)	BA-82-2022	\$15.25/pack of 10 \$13.25/10+ packs
TSA media tubes	BA-82-7322	\$13.75/pack of 10 \$12.60/10+ packs
TSA Dehydrated media	BA-78-8420	\$17.95/100 g
Parafilm 2 in. x 250 ft.	BA-71-3044	\$17.95 each

**Teacher Note:** Isopropyl (rubbing) alcohol can be purchased at any grocery store; Q-tips (sterile in the box, or can be sterilized in a test tube, stoppered by nonabsorbent cotton) could be exchanged for the Cotton Tip applicators; glass pipettes or stirring rods can be melted into a hook shape by a Bunsen burner.

**Life Science Products**

Call for current prices 1-800-245-5774 [www.lifesciprod.com](http://www.lifesciprod.com) [lspl@lifesciprod.com](mailto:lspl@lifesciprod.com)

Item	Ordering Number	Price
60x15mm Petri dish (500/case)	LS-6606	\$48.70
Red 12"x24" Biohazard Bags (200/pack)	LS-4812-R3	\$32.50
Sterile Cotton-Tipped Applicator Swabs (100/pack)	AP-4304	\$6.90

**Fisher Scientific** Call for current prices 1-800-766-7000

<b>Item</b>	<b>Ordering Number</b>	<b>Price</b>
Dehydrated LB agar	DF0140-15-4	\$61.99

## **Lab #2: The Need for Sterile Technique**

### **Introduction:**

The problem of sterile technique has already been introduced as one that constantly faces the microbiologist in the lab. Despite the need for careful attention to this detail, it is easy to become lax in this very important facet of lab work. After several successful manipulations of microorganisms, it is not uncommon to let up your guard and then have a series of contaminated labs. Every surface, every drop of fluid, every portion of the air around you is teeming with microbial growth. Only after you realize that you are living in a seething sea of life, can you fully appreciate the necessity for aseptic, or sterile, technique. As with any new knowledge, no amount of talking or reading about a topic will impress you as much as first-hand experience.

Many buildings are “closed systems” utilizing filtered air from a central heating and air conditioning unit. The air in such a building should have an abnormally low number of air-borne microorganisms compared to the air in rooms not part of a central system. For this reason, today’s lab will require that your group seek out several different types of environments within or outside of the building. When working within your classroom or school building, consider how much the air might be stirred up, not circulated very well or how much congregations of people will effect the number of microorganisms in the air.

**Problem:** Read the Introduction and the Procedure. Then describe the purpose in doing this lab. Also, identify the independent and dependent variables of the experiment.

**Hypothesis:** In the space below, describe the expected outcome of this experiment. Include how and why the independent variable(s) affects the dependent variable(s).

**Materials:**

2 sterile Petri dishes of TSA	sterile TSA for pouring	parafilm
unsterilized Petri dish	tube of sterile nutrient broth	test tube brush
5ml sterile pipette	fine-tipped marking pen	tape
sterile empty test tube	tube of sterile TSA	

**Procedure:**Part 1: Sterile TSA in a Clean but Unsterile Petri

1. Wash the non-sterile Petri dish and lid until thoroughly clean. Dry the lid and dish with a paper towel. Use the marking pen to write your name, the date, MSL#2 (for Microbiological Skills Lab #2) and “clean/not sterile” around the edges of the bottom of the dish.
2. Get a test tube of sterilized agar from the water bath. Check to be certain that the TSA is liquefied. If it is still solid or semi-solid, tell your teacher. If the agar is liquid, then remove the cap, and pour in enough agar to cover the bottom of the dish. Replace the lid of the Petri dish. After everybody in your group has poured a plate, pour the remaining agar down the drain and wash plenty of hot water down behind it. Rinse and clean the test tube. Leave the Petri dish where it is until the agar has solidified.

Part 2: Collecting Microorganisms from the Air

1. On the bottom of one of the Petri dishes that had the agar poured for you, write your name, the date, MSL#2 and your room number. Take the lid off this dish and leave the agar exposed to the air around your work area for about 30 min.
2. On the bottom of one of the Petri dishes that had the TSA poured for you, write your name, the date, MSL#2 and the location where you think that you can collect some interesting microorganisms from the air.
3. Go to the location where you think that you can collect some interesting microorganisms from the air. Some suggestions might be where there is a lot of activity, the cafeteria, a locker room, outside or a rest room. Take the lid off of this dish and leave the agar exposed to the air for about 30 minutes. Go on the next section while waiting for the 30 minutes to pass.

Part 3: Using a Pipette to Transfer Broth Without Sterile Technique

1. While waiting for the 30 minutes to pass, transfer 5 ml of nutrient broth to the sterilized and empty test tube **without** taking the normal precautions. That is, do not flame the mouths of the tubes or the end of the pipette. Next, transfer 2 ml from the second tube back to the first in the same fashion and using the same pipette. Continue to transfer 2ml back and forth 5 times without using sterile technique. Do not hurry through this procedure.
2. When you are done, cap the tubes, write your name, the date and MSL#2 on two pieces of tape and put a piece of tape on each of the two test tubes. Place the test tubes in the test tube rack in the incubator.

Don't Forget the Petri Dishes

1. After 30 minutes, retrieve the Petri dishes that you left around the school. Replace the lids on the Petri dishes, parafilm it and stack all three Petri dishes on their lids in the incubator.

**Results:**

1. Numbers of individual colonies on the Petri dishes.

Clean, but not sterile: \_\_\_\_\_

Classroom: \_\_\_\_\_

Other Location: \_\_\_\_\_

2. Numbers of different kinds of colonies on the Petri dishes.

Clean, but not sterile: \_\_\_\_\_

Classroom: \_\_\_\_\_

Other Location: \_\_\_\_\_

3. Look at the broth that you transferred with the pipette. Is there anything on the surface or at the bottom of the test tube? Is the broth clear or cloudy? Describe the appearance of the broth.
4. Swirl the broth in the test tube. Does this change the appearance of the broth? If so, it what way?

**Analysis/Conclusion:**

1. Compare the numbers and diversity of colonies with other groups. In the space below, use class data to evaluate your hypothesis.
2. What are some factors that might have affected the outcome of this lab and how can they be controlled?

3. There are several different locations that bacteria might be in liquid culture (Results, #3 and #4). Give an explanation for the locations of the bacteria in each of the different scenarios.

On the surface of the broth, only:

On the bottom of the test tube, only:

Diffused evenly throughout the broth:

Name \_\_\_\_\_

Microbiological Skills Lab #2, Part 2

## Bacterial Colony Morphology

### **Introduction:**

In the Isolation Techniques lab (MSL #1), you learned how to distribute bacterial cells so thinly that a single cell would give rise to a colony of many millions of individuals, each colony a clone of the colony's progenitor. Question #2 in the Results section of that lab asked you to describe the appearance of the 2 sets of colonies. In this lab, you will be using the official terms for describing the shapes of the colonies that you have collected from the environment. See how many of the different characteristics described in this lab you observed in that lab!

Morphology refers to the outward appearance of an organism or part of an organism. Because bacteria and other microorganisms are so small, sometimes the morphology of an organism's colony can be helpful when trying to differentiate between different species. Colony morphology can vary, however, depending on the growth medium (agar) and other environmental conditions. This variability in colonial morphology can cause confusion if you forget about it and it can also be a tool that you can use. If you are searching for a certain type of bacteria and you are familiar with its response to certain media or conditions, then you can use this information to your advantage.

Colony recognition is a personal accomplishment. The information in this lab tells you where to look for characteristics, but you must recognize the differences in the characteristics, if this is to do you any good. Also, any characteristic of a colony that enables you to differentiate it is a good characteristic to use. The characteristics provided are meant as a guide. Use whatever works for you and remember, this might be different from what works for others.

**Purpose:** After reading the Introduction and the Procedure, explain the purpose in doing this lab in the space below.

**Materials:**

fine-tipped marking pen

magnifying glass

dissecting microscope

**Procedure:**

1. Under no conditions should you ever open a Petri dish to get a better view of the morphology of a colony!
2. Use the diagrams or websites as described by your teacher to describe the morphologies of the bacteria that you collected in the last lab.
3. In the space below make a table for organizing the information that you will be collecting. Make a place on the table to identify each of the different types of colonies by a number.
4. Using the naked eye, a magnifying glass or a dissecting microscope, carefully observe each of the colonies on the Petri dishes. Always observe them through the lid of the dish. Once you have written each of the various characteristics on the table, assign a number to the colony and write that number on the lid of the dish over the colony.
5. Check the types of colonies that other groups have collected. See if you can find each of the characteristics shown on the diagram in the colonies the class has collected.

**Results:**

**Points to Ponder:**

1. Why were you instructed not to open the Petri dishes?
2. Which were the most common characteristics that the class found?
3. Which characteristic(s) was not found?
4. Which location provided the class with the greatest diversity? Why do you think that this is the case?
5. Considering #5, should you be concerned about the diversity of organisms? Why, or why not?

## Answers to Students' Questions

### Part 1

#### **Analysis/Conclusion:**

1. Compare the numbers and diversity of colonies with other groups. In the space below, use class data to evaluate your hypothesis.

*Be sure that students substantiate their hypotheses with actual data collected in this experiment.*

2. What are some factors that might have affected the outcome of this lab and how can they be controlled?

*Accept any reasonable answer. Here are some suggestions, should the students be stumped.*

*Sterile TSA and clean, but unsterile, petri: Chlorine from the tap water may have killed bacteria. Soap used to clean the plates may have washed away all bacteria. Heat from the liquid agar may have killed bacteria.*

*Collecting Microorganisms from the Air: Filtered air may have removed bacteria, lid left off too brief of a time to collect many bacteria, not enough air circulation to allow bacteria to fall on plate.*

*Using a Pipette to Transfer Broth Without Sterile Technique: Filtered air may have removed bacteria, lid left off too brief of a time to collect many bacteria, not enough air circulation to allow bacteria to fall on plate.*

3. Look at the four different locations that bacteria might be in liquid culture (Results, #3). Give an explanation for the locations of the bacteria in each of the different scenarios.

On the surface of the broth, only:

*Bacteria may require oxygen and so grow only at the surface.*

On the bottom of the test tube, only:

*Oxygen inhibits bacteria growth and so they are found only away from the surface, or the bacteria do not fully separate after dividing and so they form large clumps and sink.*

Diffused evenly throughout the broth:

*Bacteria have sufficient oxygen to feed through the media and they do not stay attached following division, so they do not easily sink, or they may have flagella to swim.*

## Part 2

### **Points to Ponder:**

1. Why were you instructed not to open the Petri dishes?

*Because unknown, and potentially pathogenic, bacteria may have fallen on the dishes and grown to huge numbers they may pose a serious health hazard.*

2. Which were the most common characteristics that the class found?

*Varies according to class data.*

3. Which characteristic(s) was not found?

*Varies according to class data.*

4. Which location provided the class with the greatest diversity? Why do you think that this is the case?

*Varies according to class data. Accept any reasonable explanation for the diversity.*

*Possible explanations include food, habitat, possibility to have a many bacteria introduced from a variety of sources, etc.*

5. Considering #5, should you be concerned about the diversity of organisms? Why, or why not?

*Accept any reasonable answer.*