

Lab #1: Isolation Techniques and Use of Petri Dish Cultures

Summary: Students are introduced to sterile technique for handling microorganisms safely in the classroom. Skills introduced include transfer of microbes from liquid culture to agar, isolating single colonies from the crowd, and creating a lawn, or carpet, of microbes.

Grade Levels: 9-12

Prior Knowledge: This is a skill lab, so the students don't have to know a darned thing about bacteria other than some are agents of disease and so safety is important. Also, knowing that bacteria are extremely small and what they see will be colonies, or very large clusters of bacteria containing potentially millions of individual cells.

Materials: In all labs in this series, the agar tryptic soy agar is used. This is only one of several that are suitable. Others, such as Nutrient Agar or Luria 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 means 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.

15 inoculating loops 15 Bunsen burners 15 bent glass rods
15 dropper bottles of 70% isopropanol (rubbing alcohol)
15 test tube racks 15 fine-tipped marking pens 15 microscope slides
15 150ml beakers

Test tube containing a mixed broth culture of *Enterobacter aerogenes* and *Serratia marcescens*.

sterile cotton tip applicator/person

4 TSA plates/person, or 120 for a class of 30

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

Outline:

1. Students are required to read the Introduction and Procedure of the lab so they can describe the objectives, or purpose, in doing this lab.
2. Students are introduced to basic safety precautions and procedures for working with microbiological material.
3. Students are taught sterile technique for handling broth and agar cultures.
4. Students are taught how to separate a mixed culture of bacteria.
5. Students are taught how to create a carpet, or lawn, of bacteria.
6. Students are asked to describe colony morphology.

Teacher Instructions:

- Using the pinky to hold the cap of the test tube is an important skill that the students must use.
- Do not allow them to set the cap on the tabletop.
- Do not let the students set the lid of the petri dish off to one side of the dish.
- Always have students dispose of their Petri dishes in the biohazard bag.
- Cultures of *E. aerogenes* and *S. marcescens* should be stored in a **non**-frost free freezer because repeated freeze/thaw cycles will kill the bacteria. If you do not have access to such a freezer, store them in a refrigerator not used for food storage.
- 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)

H₂O 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, use the above recipe, but omit the agar. Broth can be sterilized in half-filled and stoppered test tubes.
- Inoculate tubes of broth containing both *E.aerogenes* and *S. marcescens* several days prior to this activity for student use. Incubate the tubes overnight at 37°C. The tubes should be gently swirled prior to use to mix the bacteria with the broth. Be sure to make separate test tubes for each class.

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:

Materials Price List/Ordering Information:

Carolina 1-800-334-5551 www.carolina.com

Item	Ordering Number	Price
Bacterial Spreader	BA-21-5820	\$2.70 each
Inoculation Loop	BA-21-5826	\$1.80 each
Enterobacter aerogenes	BA-15-5031	\$7.50/tube
Serratia marcescens	BA-15-5452	\$7.50/tube
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. 60 x 15 mm Standard petri dishes (500/case) can be purchased through Life Science Products (1-800-245-5774) for \$48.70.

Life Science Products

Call for current prices 1-800-245-5774 www.lifesciprod.com 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

4 TSA plates/person	test tube rack	fine-tipped marking pen
parafilm and scissors	microscope slide	150ml beaker
paper towels	sponge	matches
biohazard bag		

Test tube containing a mixed broth culture of *Enterobacter aerogenes* and *Serratia marcescens*.

Safety:

1. A microbiology lab is potentially a very dangerous place. For this reason it is extremely important that you follow all safety guidelines and always practice sterile technique when handling microbes, unless instructed otherwise.
2. There should be no books or papers at your workstation except this lab packet.
3. Never have any food or drink at your workstation.
4. Always thoroughly wash your hands with disinfectant soap or alcohol before leaving your workstation.
5. Never open a Petri dish after you have inoculated it and allowed it to incubate overnight.
6. Always dispose of used material in the biohazard bag, unless instructed otherwise.

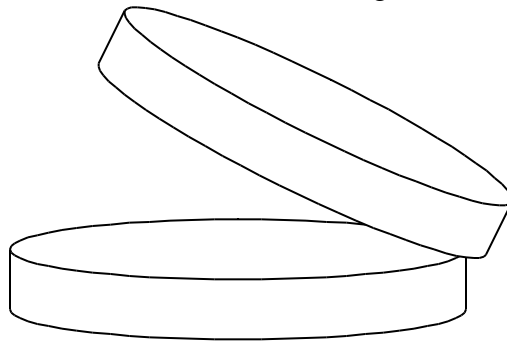
Procedure:

1. Wash an area on the tabletop larger than the area that you will be using, first by wiping with a damp sponge. Then, pour a small amount of 70% isopropanol on the surface and wipe it around with a clean paper towel. Use the paper towel to wipe both of your hands as well as each finger individually. **Be sure to allow the alcohol at your table time to completely evaporate before lighting any flames!** Throw the paper towel in the trash before going on to the next step.
2. After there is no indication of alcohol fumes at your table, light the Bunsen burner. Adjust the flame so there is a small blue cone at the bottom of the flame.

Technique #1: Streak Plate Method of Isolation

1. Sterilize the inoculating loop by holding it at the top of the small blue cone in the flame of the Bunsen burner until the loop is red-hot. Remove the loop and allow it to cool for 10-15 seconds.

2. While holding the inoculating loop between your thumb and forefinger(s), use your pinky to remove the cap of the test tube containing the broth of mixed cultures of *E. aerogenes* and *S. marcescens*. Do not set the cap on the tabletop.
3. While keeping the test tube of broth held at an angle, pass the mouth of the test tube through the flame 2-3 times.
4. Slowly insert the sterilized loop into the mouth of the test tube until you reach the broth. Do not plunge the loop deep into the broth, because only the tip is sterile and you can contaminate the culture.
5. Once you have some broth on the tip of the inoculating loop, remove the loop. Flame the mouth of the test tube as you did in step #3 and replace the cap. Set the culture in the test tube rack.
6. Lift one side of the lid of a Petri dish to form a 45° angle (see the drawing below).



7. Gently wipe the inoculating loop across the surface of the tryptic soy agar (TSA) using one of the patterns shown below. Be careful not to gouge the surface of the agar or dig it up in any way.
8. Sterilize the inoculating loop as you did in step #3. Set the loop down on a pen with the hot end sticking out past the pen so the pen and the tabletop are not damaged.
9. Use the marking pen to write in small letters around the bottom edge of the Petri dish: the date, your name and MSL#1-streak. MSL#1 stands for Microbiology Skills Lab #1. This will tell us the organisms that we hope to grow. Cut a _ square of parafilm and wrap it around the edge of the Petri dish to slow evaporation.
10. Allow your lab partner(s) to practice this technique.

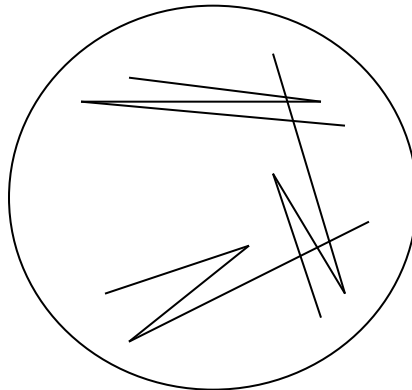
Technique #2: Spreading Bacteria with a Bent Glass Rod.

1. Use the same techniques as you did in the preceding section to sterilize the inoculating loop and place a single loopful of mixed culture broth near one edge of a Petri dish. Replace the lid of the dish, but do not parafilm it yet. (Don't forget to sterilize the loop and set it down before doing anything else!).
2. Place 2-3 drops of 70% isopropanol on the part of the bent glass rod that you will be using to spread the broth.
3. Light the alcohol by passing the glass rod through the flame of the Bunsen burner. Allow the alcohol to burn completely. Warning: Do NOT hold the glass rod in the flame of the Bunsen burner as you did in the first technique because the glass will break.
4. Repeat steps # 2 and #3.
5. Lift one side of the Petri dish lid 45° and place the hot end of the glass rod on the agar, but not on the drop of broth. The agar may sizzle briefly. After a short period of time the glass rod should be cool.
6. Use the glass rod to spread the drop of broth over the entire surface of the TSA agar. You may need to lift the lid slightly above the dish to get the rod in to the agar. If you do, keep the lid over the agar, so no contaminants fall on to the agar from the air.
7. After wiping the drop across the agar's surface, replace the lid on the Petri dish and repeat steps #2 and #3. Place the glass rod on the pen to prevent damage to the tabletop.
8. Use the marking pen to write in small letters around the bottom edge of the Petri dish: the date, your name and MSL#1-rod. Cut a _ square of parafilm and wrap it around the edge of the Petri dish to slow evaporation.
9. Allow your lab partner(s) to practice this technique.

Technique #3: Isolation Using a Cotton Swab

1. Pour enough 70% isopropanol into the 150ml beaker to cover the bottom.
2. While holding the cotton swab between your thumb and forefinger(s), use your pinky to remove and hold the cap from the test tube containing the mixed cultures of *E. aerogenes* and *S. marcescens*.

3. Flame the mouth of the test tube and while holding it at an angle; immerse only the tip of the cotton swab into the broth.
4. Remove the cotton swab from the test tube, flame the mouth of the test tube, replace the cap and set the test tube back on the rack.
5. Lift the lid of the remaining unused Petri dish at a 45° angle and place a drop of broth near the edge of the plate.
6. Place the cotton end of the swab in the 150 ml beaker.
7. Sterilize the inoculating loop. After allowing the loop to cool for 10-15 seconds, lift the lid of the Petri dish 45° and make a single swipe with the inoculating loop through the drop that you just made with the cotton swab. Without lifting the loop from the surface of the agar, make a second series of zigzags next to where the cotton swab was wiped on the agar. Close the lid of the dish.
8. Sterilize the loop and repeat step #7, making a single pass through the most recent set of zigzags, rather than where the cotton swab was. See the diagram below, if necessary.



9. Sterilize the inoculating loop. Set the loop down with the hot end over a pen or sink, so the tabletop is not damaged.
10. Use the marking pen to write in small letters around the bottom edge of the Petri dish: the date, your name and MSL#1-swab. MSL#1 stands for Microbiology Skills Lab #1. This will tell us the organisms that we hope to grow. Cut a _ square of parafilm and wrap it around the edge of the Petri dish to slow evaporation.
11. Allow your lab partner(s) to practice this technique.

Streak Plate

Glass Rod

Cotton Swab

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2. In the space below, describe the differences in appearance of the two different types of bacterial colonies that you have grown on the plates.
3. Which of the three techniques worked best to isolate a single colony? Why?

Answers to Students' Questions

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

The purpose in doing this lab is to learn the safe handling of bacteria in the laboratory setting and to learn some techniques to transfer and isolate bacteria.

Reflections on the Lab:

1. Aside from the three techniques formally presented in this lab, what other skills did this lab introduce to you?

This lab also introduced (or, reinforced important safety procedures, such as never eating at the lab station, washing hands and tabletop, never to open a Petri dish after it has been cultured, and disposal in biohazard. In addition, this lab asked us to observe differences in colonies.

2. What is the value of each of the additional skills described in #1?

The safety procedures are valuable to protect the health of those handling microorganisms. Observation skills are important in describing differences between various organisms.

3. What techniques would you need to know in order to set up or break down this lab for other students?

Other techniques that are important to know include preparation, sterilization and transfer of the media used. Also, the safe disposal of the biohazard bag after the lab would be important to know.

Results:

2. In the space below, describe the differences in appearance of the two different types of bacterial colonies that you have grown on the plates.

Differences might include color, texture, elevation, sheen, or a description of the edges (margins) of the colonies.

3. Which of the three techniques worked best to isolate a single colony? Why?

Either the streak plate or the cotton swab should have work best. This is because a small amount of bacteria was placed on the agar and then dragging the sterilized loop through the broth spread the bacteria out over a wide area.

4. Evaluate your technique in working with microorganisms.

Accept all reasonable self-evaluations.

5. How can you improve your microbiological lab technique?

Like any skill, practice and careful observation of technique is the best way to improve.

6. How are the techniques presented in this lab important in understanding microbiology?

Students will probably repeat the safety and sterile techniques described in question #1 of the “reflections” piece of this lab. In the post-lab, you may wish to use this question as a segue into the following lab, which investigates the ubiquity of microorganisms in the environment.

7. What should we do next to better understand microorganisms? Why?

Accept any reasonable answer. The purpose of this question is two-fold. It is to encourage students to think about what they know and don't know and it is to get them to explain why they make the statements they do.