

Teacher Guide: Sterility & Aseptic Technique

Goals

In this lab activity, students will:

- Learn about sterility and how to employ aseptic technique
- Formulate a testable hypothesis
- Explore microbial diversity from various environments

Learning Objectives

Upon completion of this activity, students will (i) understand that bacteria can be found in a wide range of environmental habitats; (ii) be able to use aseptic technique when working with microbes; (iii) understand how scientists culture bacteria based on nutrient requirements; and (iv) identify *Wolbachia* as an unculturable microbe.

Prerequisite Skills

No prerequisite skills are required for this activity. If your class has not covered how to formulate a hypothesis and identify controls/variables, Pre-Lab Activity 1 can be completed as a class discussion.

Group Size

This activity can be performed in small groups (2-4 students) or as an individual project.

Teaching Time

The entire lab will take approximately one week. Bacterial colonies will grow faster in a warm incubator and slower at room temperature. In either case, growth should be visible within 3-5 days. To optimize class time, pour the plates on Friday and streak the plates on Monday.

- Day 1 (full class period): Pour plates; complete pre-lab activity
- Day 2 (full class period): Swab environmental surfaces and streak the plates
- Days 3-5 (partial class periods): Briefly record observations; during remaining class time, complete post-lab activity (colony morphology research)

Supplies

We recommend colored pencils for Pre-Lab Activity 2. Microbiology supplies for this lab are commonly available from most scientific vendors. We recommend purchasing Nutrient Agar for general bacterial growth. Vendors typically sell powdered agar, bottled agar, and prepared agar plates.

Type of Media	Cost	Shelf-Life	Manual Effort & Class Time Required to Prepare Plates
Powdered agar	Low	High	High
Bottled agar *	Medium	Medium	Medium
Prepared agar plates	High	Low	None

* The lab activity is based on this type of media

If you would like students to design their own experiments, additional types of media (i.e., Blood Agar, Potato Dextrose Agar, etc.) may be purchased to cultivate specialized microbes. Depending on your specific goals for this activity, you may wish to provide control plates with anticipated bacterial colonies.

Pouring Plates

Refer to the vendor's product information guide for best practices. In general, pour plates in a draft-free area, laminar flow hood, or biosafety cabinet. Clear the surface and wipe with 70% ethanol or 70% isopropyl alcohol. Place Petri dishes in a single row or short stacks of 3. To pour agar into the bottom (deeper portion) of the Petri dish, gently lift off the lid (thin, shallow portion) and fill 2/3 with agar. Place the lid back on the plate and gently swirl to coat the bottom. Allow plates to completely cool (about 1 hour) and use immediately or store upside down in the refrigerator (4°C) for 2-4 weeks.

Sterile Cotton Swabs

For best results, purchase sterile cotton swabs that are individually wrapped. If sterile swabs are unavailable or beyond the allocated budget for this experiment, you may use over-the-counter swabs. In this case, students will be introducing a variable (unsterilized swab) to the experiment. We recommended swabbing a plate with only the unsterilized cotton as a *negative* control. If students obtain growth from the negative control, this indicates that similar microbes on the experimental plates may have originated from the swab rather than their environmental surface.

Incubator vs. Room Temperature

We recommend incubating plates overnight in an incubator at 37°C. You may also place plates in a warm location of the classroom. Colder environments will likely result in slower growth rates; temperature could be a fun variable for students to explore! In this case, perhaps the variables to be tested could be environmental conditions (such as temperature, light, humidity) rather than different environments.

Safety

Always wear gloves and wash hands before and after each activity. Treat cultured microbes as if they are potential pathogens; do not allow students to open the agar plates or touch the colonies.

Disposal

Cotton swabs and unused plates may be placed in the regular trash. We recommend sealing all swabbed plates with Parafilm. When the experiment is complete, place plates in an autoclave bag and sterilize for ~15 min. If you do not have access to an autoclave, use a 20% bleach solution or 70% ethanol solution to kill bacteria and then dispose in the regular trash.