Practicing Aseptic Technique with Microbial Cultures

Introduction

Working with bacterial cultures educates students in differences in bacterial shape, composition and incubation temperture. All bacterial cultures sold by Flinn Scientific® are nonpathogenic. However, it is extremely important to always practice aseptic technique to avoid culturing unwanted microorganisms obtained from the environment.

Concepts

- Aseptic technique
- Microorganisms
- Microbiology

Background

Microorganisms found in their natural environment typically exist with other microorganisms and not as a single species. For example, a small sample of pond water or soil is likely to contain many different types of bacteria, viruses, yeast, molds, algae and protozoa.

When studying microorganisms in the laboratory the most accurate data is obtained when studying only one species at a time. Various types of microorganisms can be isolated to obtain what is known as a *pure culture*. A pure culture contains only one *genus* and *species* of organism. When isolating an organism for research purposes it is important that the pure culture is not contaminated. A pure culture can become contaminated by other bacteria from sources such as the air, skin or the lab table.

To minimize the risk of contamination, a method known as *aseptic technique* is practiced. Aseptic technique is a procedure designed to keep unwanted microorganisms from contaminating sterile materials or pure cultures of microorganisms. Not only can unwanted species contaminate a pure culture and yield incorrect results but they also may be pathogenic and facilitate disease growth. Therefore the prudent practice of aseptic technique is extremely important when working in the microbiology laboratory.

Materials

Bunsen burner
Disinfectant such as Lysol®
Inoculating loop
Microbial culture
Sterile plate with nutrient agar

Safety Precautions

Always practice aseptic technique when working with bacteria. Use caution when working with a Bunsen burner, the lip of the culture tubes may be hot. Wear chemical splash goggles, chemical-resistant gloves and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all laboratory safety guidelines. Please review current Material Safety Data Sheets for additional safety, handling and disposal information.

Procedure

1. Thoroughly disinfect the lab table by applying a disinfectant such as Lysol and allow it to air dry.
2. Obtain an inoculating loop and a culture tube containing a microorganism.
3. Light the Bunsen burner and adjust to obtain a blue cone flame.
4. Loosen the culture tube cap but do not remove it.
5. Pass the inoculating loop through the flame causing it to become red hot (see Figure 1). *Note:* The tip of the loop is sterilized when red hot. Do not wave the loop back and forth or blow on it to cool it more quickly. Such action will increase the likelihood of airborne microorganisms adhering to the loop and contaminating the specimen.

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6. Remove the tube cap and hold it between your little finger and ring finger of the hand holding the loop (see Figure 2).
7. Briefly flame the lip of the tube while rotating the tube to ensure no microorganisms remain on the lip (see Figure 3).
8. Dip the inoculating loop into the culture. *Note:* If the tip is placed in the culture media while it is still hot it may kill the microorganisms. If a sizzling sound is heard when the loop enters the media, the loop is too hot; repeat steps 5–8.
9. Briefly reflame the culture tube lip again, rotating the tube; recap and set aside.
10. Lift the lid of the nutrient agar plate, as little as possible, and spread the bacteria on the inoculating loop over the surface of the agar (see Figure 4). Rotate the Petri dish 90° and repeat.
11. Reflame the inoculating loop until red hot.
12. Thoroughly disinfect the lab table as directed in step 1.

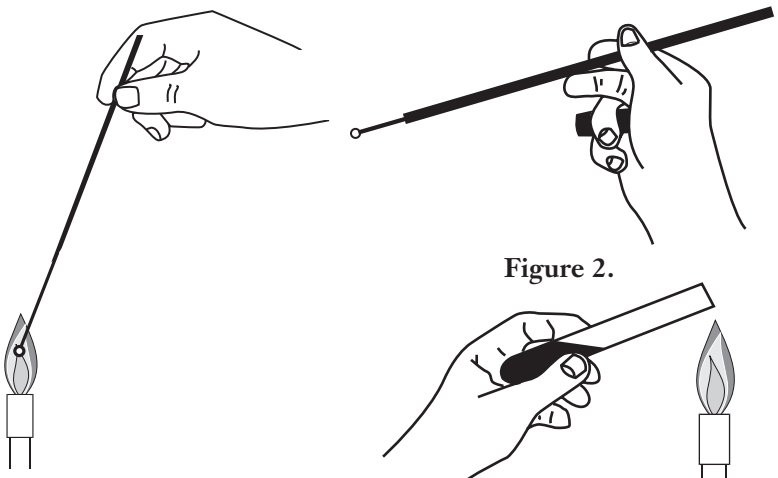

Figure 1. Flaming an inoculating loop.

Figure 2.

Figure 3. Flaming a test tube.

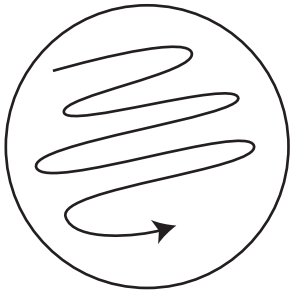

Figure 4.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory wastes. All microorganisms purchased from Flinn Scientific are non-pathogenic. However, to be safe all bacterial cultures should be considered pathogenic and destroyed according to Flinn Biological Waste Disposal Type I before disposal in the trash. The best way to dispose of bacteria on agar plates is to autoclave them in a heat-stable biohazard bag. If an autoclave is not available an alternative is to bleach the plates. Saturate the agar plates with a 10% household bleach solution. Allow plates to sit for 24 hours before disposing.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Form and function

Content Standards: Grades 5–8
Content Standard C: Life Science, structure and function in living systems, reproduction and heredity, regulation and behavior, population and ecosystems, diversity and adaptations of organisms

Content Standards: Grades 9–12
Content Standard C: Life Science, the cell, behavior of organisms

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Tips

- In this activity microbial cultures are transferred from a culture slant to a culture plate. They may also be transferred to another slant. Using the proper agar and incubation conditions are the most critical variables in microbial growth success.
- The nutrient agar plates used in this activity may be purchased as sterile prepared plates. Dehydrated nutrient agar and sterile plates may be purchased and prepared by the instructor using a hot plate and an autoclave as an economical alternative.
- Many students find it helpful to see the instructor demonstrate this technique to reinforce proper practice.
- Any microbial culture will work to practice aseptic technique. We recommend *Escherichia coli* as it is common and easy to work with.

References

Learning About Microbes: A Laboratory Manual; J. Weston Walch: Portland, Maine, **1998**; p 3–5.

Materials for *Practicing Aseptic Technique with Microbial Cultures* are available from Flinn Scientific, Inc.

Catalog No.	Description
LM1006	Bacterial Culture, Escherichia coli
AP1051	Inoculating Loop, Nichrome Wire
FB0526	Nutrient Agar Plate, Prepared
N0077	Nutrient Agar, Bottles
AP8170	Petri Dish, Disposable, Polystyrene, Sterile
N0020	Nutrient Agar, Dehydrated, 500 g
L0035	Lysol®, 500 mL

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

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