

E X E R C I S E

6

Aseptic Technique

OBJECTIVES

At the conclusion of the exercise, you should...

1. know the purpose of aseptic technique.
2. be able to perform aseptic technique.
3. learn how to use a Bunsen burner properly.
4. learn how to transfer bacteria from test tubes containing broth or agar.
5. learn how to transfer bacteria from Petri plates.
6. learn how to transfer bacteria from broth and agar to microscope slides.
7. be able to use aseptic technique when performing all procedures in a microbiology lab.

INTRODUCTION

Bacteria must be **cultured** (grown) in order to study them. They are **inoculated** (introduced) into culture media to grow them in order to perform the tests necessary for these studies. The inoculations have to be performed without adding other unwanted microbes (**contaminants**). The process of growing bacteria in **pure** (uncontaminated) cultures is called **aseptic** technique. This exercise will give you the guidelines for performing aseptic technique when working with bacteria, not only for keeping the cultures pure, but for safety reasons. You will use this technique with all the exercises in this manual, and will be referred to this exercise often.

MATERIALS

Cultures:

Broth cultures of bacteria
Agar slants of bacteria

Media:

Trypticase soy broth
Trypticase agar slant (optional)

Supplies:

Inoculating loop
Inoculating needle

PROCEDURES

Day 1 (inoculations)

Technical Background

Specialized tools are used when handling bacteria. The most commonly used one is the **inoculating loop**. Others are **inoculating needles**, wood sticks, glass rods, cotton swabs, syringes, **pipettes**, and **mechanical pipettors** for the pipettes. The inoculating loop and needle will be described in this exercise. The others will be introduced in later exercises.

The inoculating loop is a wire with a loop on the end, attached to an insulated handle. The needle is a straight wire on an insulated handle. They are both heated and cooled before using to transfer bacteria aseptically. The **Bunsen burner** is used to produce a flame from gas for heating.

Aseptic Technique: Transferring from a Test Tube to a Test Tube

(**Note:** Observe the demonstration by your instructor for this procedure.)

1. Turn on the Bunsen burner.
2. Adjust the flame so that there are two color cones in the flame (see diagram).
3. Heat the inoculating loop (or needle) in the inside cone until it is red hot.
4. Cool the loop by holding it in the air for a few minutes or touching a sterile area of the media or container (DO NOT wave it around).
5. Use the test tube with bacteria in a broth first. Vortex or mix the culture to distribute the organisms throughout the broth.
6. Hold the test tube in one hand and remove the cap with the ring and pinkie fingers of the hand holding the inoculating loop.
7. Pass the top of the test tube through the flame.
8. Insert the loop into the liquid.
9. Remove the loopful of liquid.
10. Pass the top of the tube through the flame again, and replace the cap.
11. Be careful with the inoculating loop that has the loopful of bacteria in it. Transfer the loopful of bacteria to the test tube containing sterile broth by removing the cap and swirling the loopful of bacteria in the broth. . Replace the cap immediately.
12. Once the transfer is complete, immediately flame the loop until it is red-hot.
13. Let it cool before putting it down.
14. Incubate the broth at 37C until the next laboratory period.
15. Optional: Inoculate an agar slant by repeating steps 3-10 and passing the loopful of bacteria back and forth over the surface of the slant.

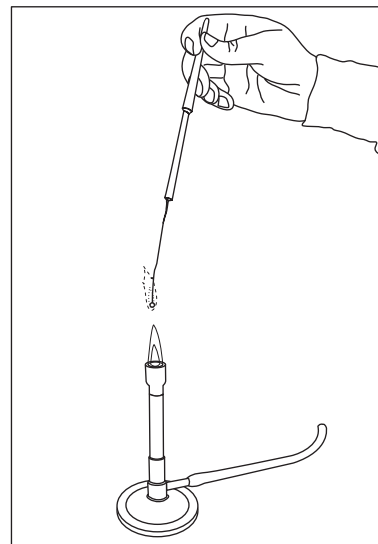
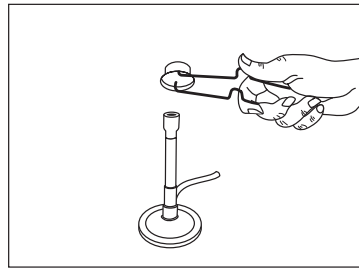


Figure 1. Flaming the loop.

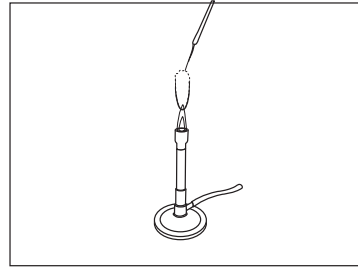
16. Incubate the agar slant until the next laboratory period.

Note: Follow Steps 1 through 10 to transfer from a test tube to a labeled microscope slide or a Petri dish with agar. These aseptic transfers will be described in the following exercises.

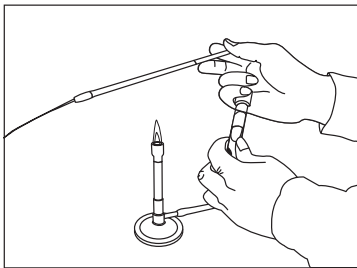
ASEPTIC TECHNIQUE: TRANSFERRING FROM A TEST TUBE



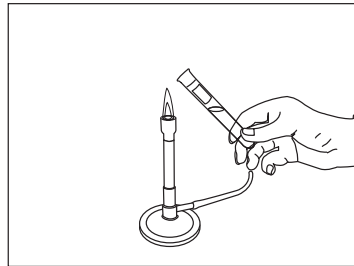
Light Bunsen burner



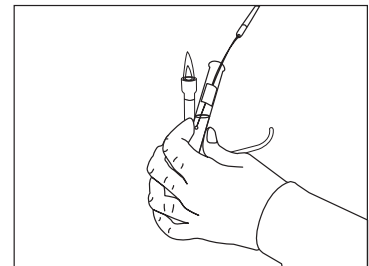
Flame loop



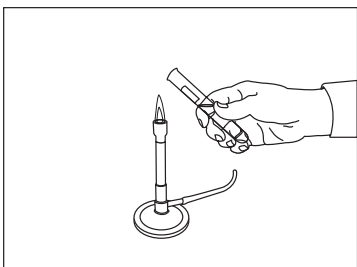
Remove cap



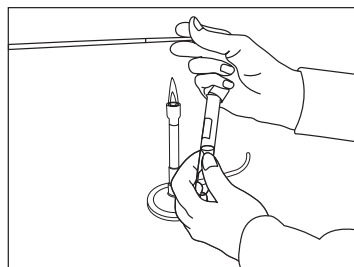
Flame test tube



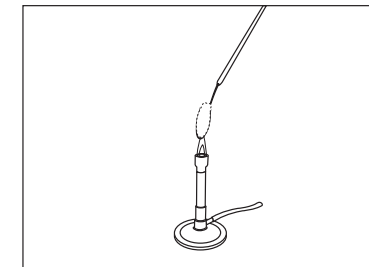
Take loopful of culture



Flame test tube



Replace cap



Flame loop

Day 2: Observations

1. Note the appearance of growth in the broth and agar slant and draw under the Data section.
2. If no growth is observed, repeat the procedure for the next laboratory period.

EVALUATION OF RESULTS (EXERCISE 6: ASEPTIC TECHNIQUE)

Purpose

Data

Draw the appearance of growth in the broth and agar slant (if applicable)

CONCLUSIONS, DISCUSSIONS, AND QUESTIONS

1. Discuss two reasons why aseptic technique is used when studying bacteria.
2. List the advantages for using an inoculating needle compared to using an inoculating loop.
3. List two reasons why there may be no growth observed after inoculating a broth or slant with an organism.
4. List the purposes for using aseptic technique.
5. Define the following:
 - Sterile culture media–
 - Inoculated culture–
 - Pure culture–
 - Contaminated culture–

