Transfer of Bacteria: Aseptic Technique

Study without thinking is worthless; thinking without study is dangerous.

CONFUCIUS

Objectives

After completing this exercise, you should be able to:

1. Provide the rationale for aseptic technique.
2. Differentiate among the following: broth culture, agar slant, and agar deep.
3. Aseptically transfer bacteria from one form of culture medium to another.

Background

In the laboratory, bacteria must be cultured in order to facilitate identification and to examine their growth and metabolism. Bacteria are **inoculated,** or introduced, into various forms of culture media in order to keep them alive and to study their growth. Inoculations must be done without introducing unwanted microbes, or **contaminants,** into the media. **Aseptic technique** is used in microbiology to exclude contaminants.

All culture media are **sterilized,** or rendered free of all life, prior to use. Sterilization is usually accomplished using an autoclave. Containers of culture media, such as test tubes or Petri plates, should not be opened until you are ready to work with them, and even then, they should not be left open.

Broth cultures provide large numbers of bacteria in a small space and are easily transported. **Agar slants** are test tubes containing solid culture media that were left at an angle while the agar solidified. Agar slants, like Petri plates, provide a solid growth surface, but slants are easier to store and transport than Petri plates. Agar is allowed to solidify in the bottom of a test tube to make an **agar deep.** Deeps are often used to grow bacteria that require less oxygen than is present on the surface of the medium. Semisolid agar deeps containing 0.5–0.7% agar instead of the usual 1.5% agar can be used to determine whether a bacterium is motile. Motile bacteria will move away from the point of inoculation, giving the appearance of an inverted Christmas tree.

Aseptic transfer and inoculation are usually performed with a sterile, heat-resistant, noncorroding Nichrome wire attached to an insulated handle. When the end of the wire is bent into a loop, it is called an **inoculating loop;** when straight, it is an **inoculating needle** (Figure 10.1). For special purposes, cultures may also be transferred with sterile cotton swabs, pipettes, glass rods, or syringes. These techniques will be introduced in later exercises.

Whether to use an inoculating loop or a needle depends on the form of the medium; after completing this exercise, you will be able to decide which instrument is to be used.

Materials

Tubes containing nutrient broth (3)

Tubes containing nutrient agar slants (3)

Tubes containing nutrient semisolid agar deeps (3)

Inoculating loop

Inoculating needle

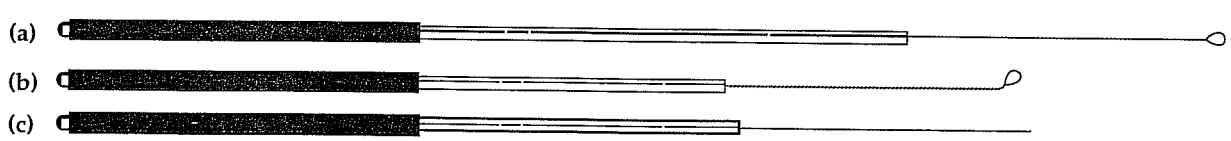


Figure 10.1

(a) An inoculating loop. (b) A variation of the inoculating loop in which the loop is bent at a 45° angle. (c) An inoculating needle.

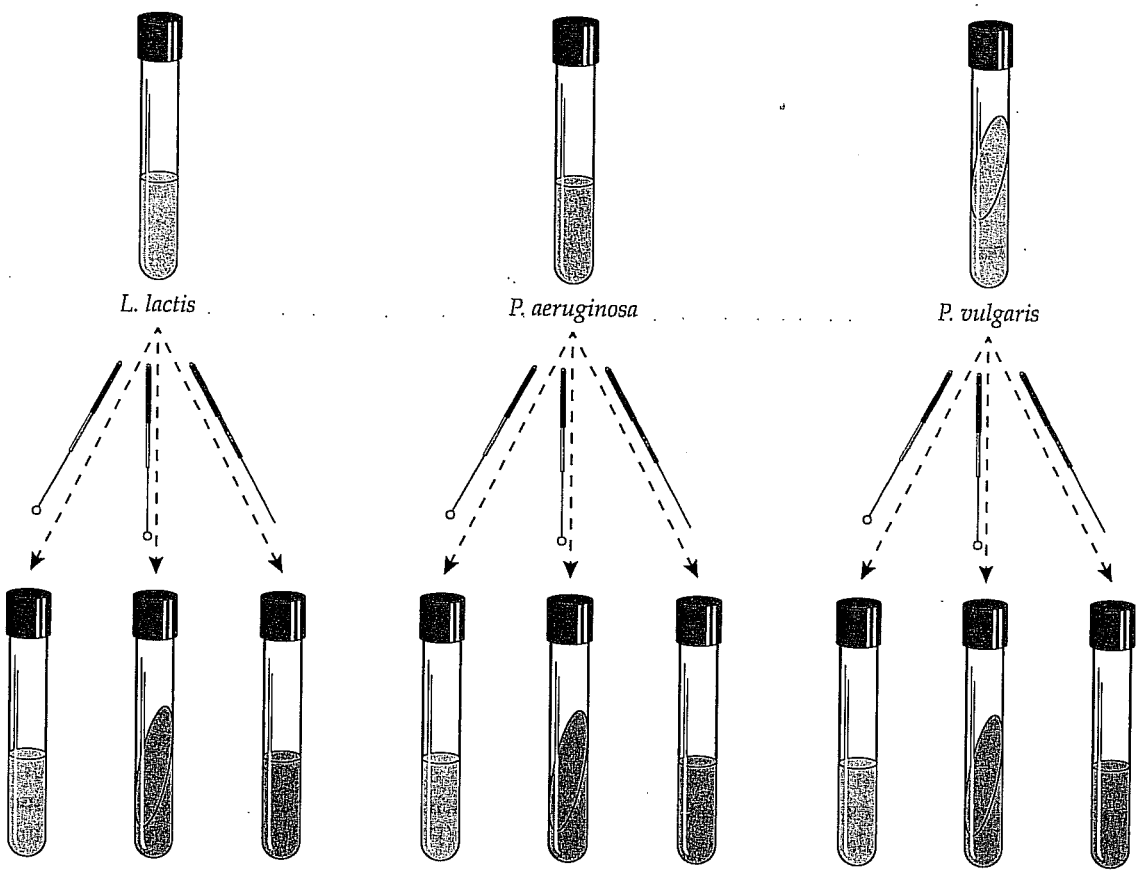


Figure 10.2

Inoculate one tube of each medium with each of the cultures using a loop or needle as indicated. Work with only one culture at a time to avoid contamination.

Test-tube rack

Gram-staining reagents

Cultures

Lactococcus lactis broth

Pseudomonas aeruginosa broth

Proteus vulgaris slant

Techniques Required

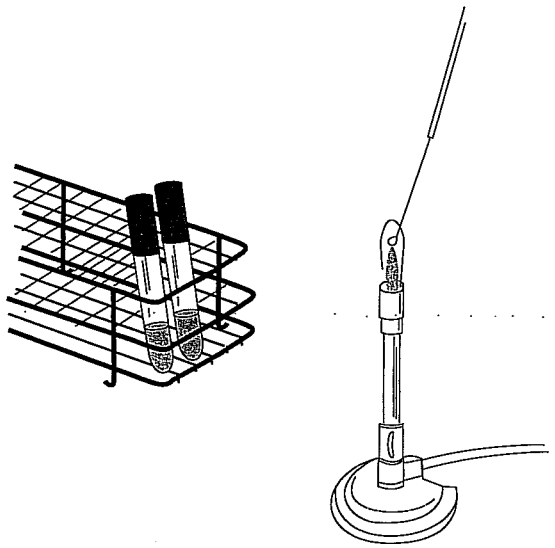
Compound light microscopy, Exercise 1

Smear preparation, Exercise 3

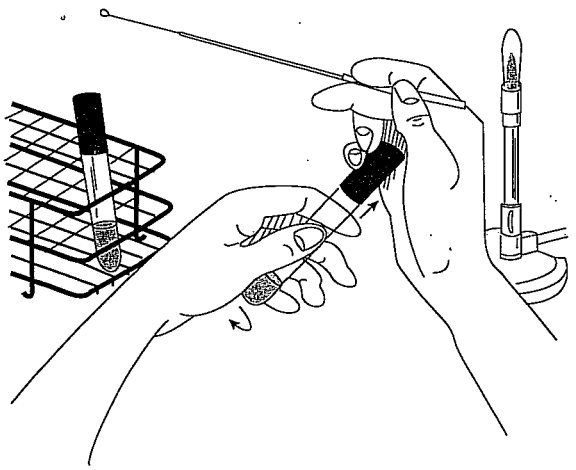
Gram staining, Exercise 5

Procedure (Figure 10.2)

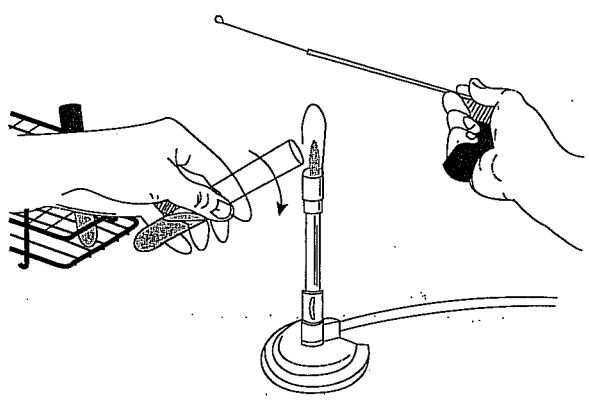
1. Work with only one of the bacterial cultures at a time to prevent any mix-ups or cross-contamination. Label one tube of each medium with the name of the first culture, your name, the date, and your lab section. Inoculate each tube as described and then work with the next culture. Begin with one of the broth cultures, and gently tap the bottom of it to resuspend the sediment.
2. To inoculate nutrient broth, hold the inoculating loop in your dominant hand and one of the broth cultures of bacteria in the other hand.
   a. Sterilize the loop by holding the wire in a Bunsen burner flame (Figure 10.3a). Heat to redness. Why? _____
   b. Holding the loop like a pencil or paintbrush, curl the little finger of the same hand around the cap of the broth culture. Gently pull the cap off the tube while turning the culture tube (Figure 10.3b). If cotton stoppers are used, simply grasp the stopper with your finger. Do not set down the cap. Why not? _____



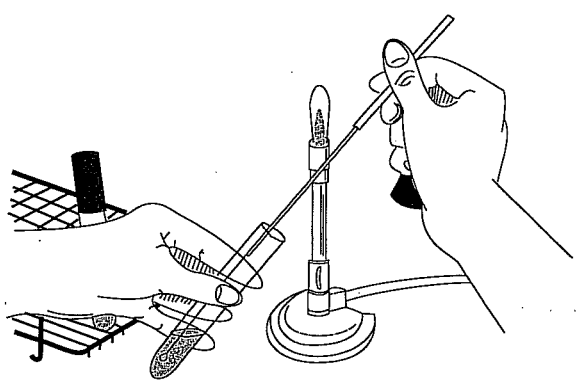
(a) Sterilize the loop by holding the wire in the flame until it is red-hot.



(b) While holding the sterile loop and the bacterial culture, remove the cap as shown.



(c) Briefly pass the mouth of the tube through the flame three times before inserting the loop for an inoculum.



(d) Get a loopful of culture, heat the mouth of the tube, and replace the cap.

Figure 10.3
Inoculating procedures.

c. Holding the tube at an angle, pass the mouth of the tube through the flame three times (Figure 10.3c). What is the purpose of flaming the mouth of the tube? _____
Always hold culture tubes and uninoculated tubes at about a 20° angle to minimize the amount of dust that could fall into them. Do not tip the tube too far, or the liquid will leak out around the loose-fitting cap. Do not let the top edge of the tube touch anything.

d. Immerse the sterilized, cooled loop into the broth culture to obtain a loopful of culture (Figure 10.3d). Why must the loop be cooled first?

_____
Remove the loop, and while holding the loop, flame the mouth of the tube and recap it by turning the tube into the cap. Place the tube in your test-tube rack.

e. Remove the cap from a tube of sterile nutrient broth as previously described, and flame the

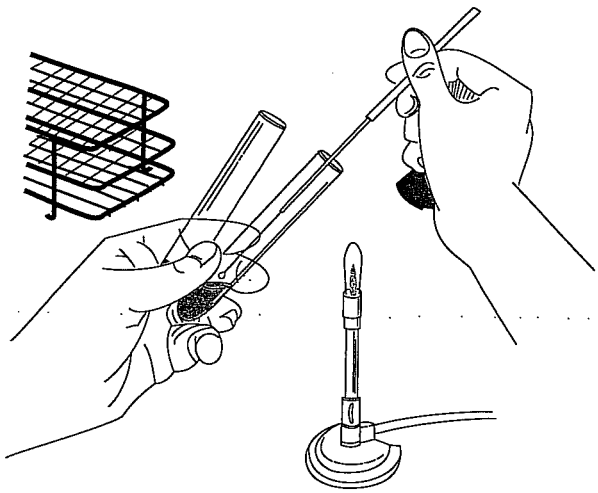


Figure 10.4
Experienced laboratory technicians can transfer cultures aseptically while holding multiple test tubes.

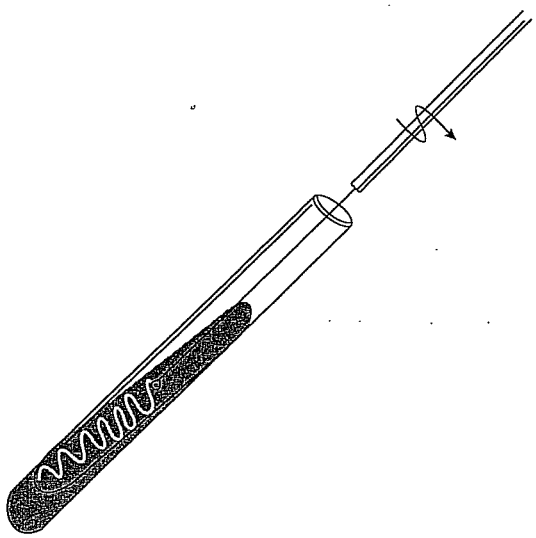


Figure 10.5
Inoculate a slant by streaking the loop back and forth across the surface of the agar.

mouth of the tube. Immerse the inoculating loop into the sterile broth and then withdraw it from the tube. Flame the mouth of the tube and replace the cap. Return the tube to the test-tube rack.

f. Reflame the loop until it is red and let it cool. Some microbiologists prefer to hold several tubes in their hands at once (Figure 10.4). *Do not* attempt holding and transferring between multiple tubes until you have mastered aseptic transfer techniques.

3. Obtain a nutrient agar slant. Repeat steps 2a–2d, and inoculate the slant by moving the loop gently across the agar surface from the bottom of the slant to the top, being careful not to gouge the agar (Figure 10.5). Flame the mouth of the tube and replace the cap. Flame your loop and let it cool.
4. Obtain a nutrient semisolid agar deep, and using your inoculating *needle*, repeat steps 2a–2d. Inoculate the semisolid agar deep by plunging the needle straight down the middle of the deep and then pulling it out through the same stab, as shown in Figure 10.6. Flame the mouth of the tube and replace the cap. Flame your needle and let it cool.
5. Using the other broth culture, label one tube of each medium as described in step 1; inoculate a broth culture, agar slant, and semisolid agar deep, as described in steps 2, 3, and 4, using your inoculating loop and needle.
6. Label one tube of each medium with *Proteus vulgaris* as described in step 1. To transfer *Proteus*

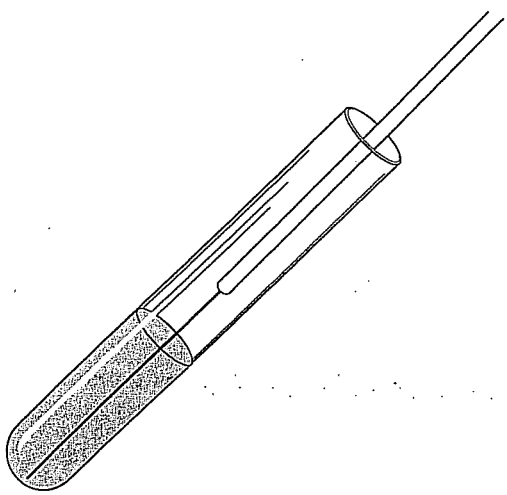


Figure 10.6
Inoculate an agar deep by stabbing into the agar with a needle.

vulgaris, flame your loop and allow it to cool. Flame the mouth of the tube, and use your inoculating loop to carefully scrape a small amount of the culture off of the agar. Do not gouge the agar. Flame the mouth of the tube and replace the cap. Inoculate a broth and a slant as described in steps 2 and 3. Inoculate a semisolid agar deep with an inoculating needle. Carefully scrape a small amount of

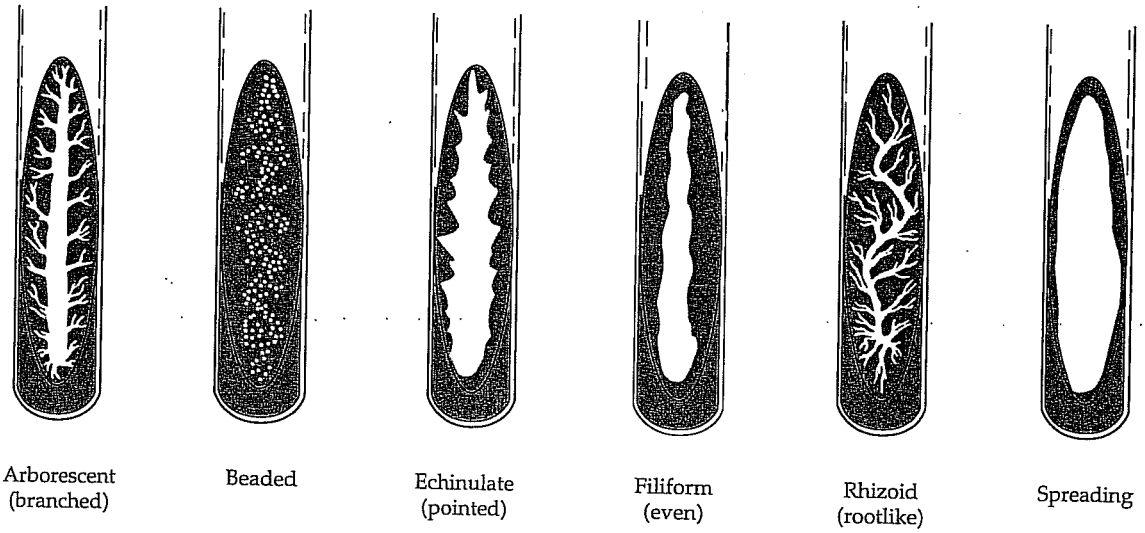


Figure 10.7
Patterns of growth on agar slants.

culture from the slant and inoculate the deep as described in step 4.

7. Incubate all tubes at 35°C until the next period.
8. Record the appearance of each culture, referring to Figure 10.7.
9. Make a smear of the *Lactococcus* broth culture and the *Lactococcus* slant culture. Perform a gram stain on both smears and compare them.

Exercise 10

Transfer of Bacteria: Aseptic Technique

LABORATORY REPORT

NAME _____

DATE _____

LAB SECTION _____

Purpose

_____

_____

_____

Data

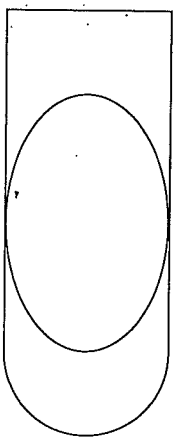
Nutrient Broth

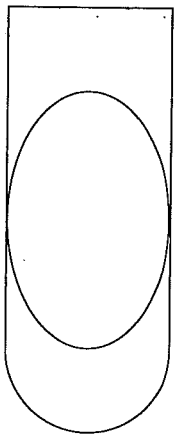
Describe the nutrient broth cultures.

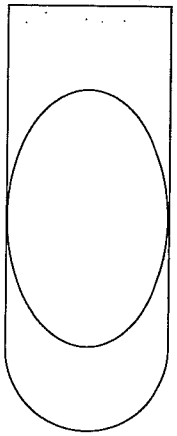
Bacterium	Is it turbid?	Is flocculent, pellicle, or sediment present?	Pigment
Lactococcus lactis			
Pseudomonas aeruginosa			
Proteus vulgaris			

Nutrient Agar Slant

Sketch the appearance of each culture. Note any pigmentation.





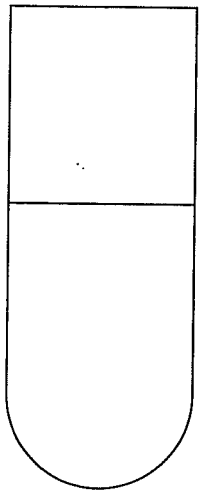


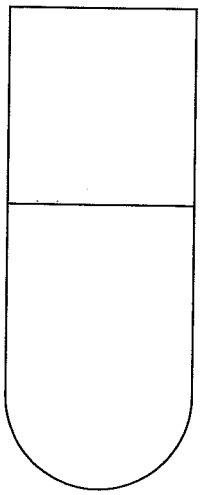
Bacteria: *Lactococcus lactis* *Pseudomonas aeruginosa* *Proteus vulgaris*

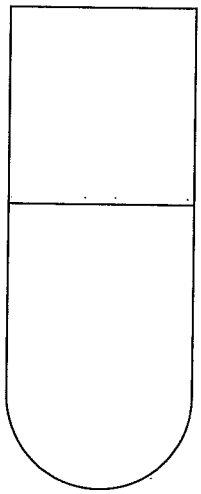
Pattern of growth: _____ _____ _____

Nutrient Semisolid Agar Deep

Show the location of bacterial growth and note any pigment formation.







Bacteria: *Lactococcus lactis* *Pseudomonas aeruginosa* *Proteus vulgaris*

Comparison of Broth and Slant Cultures

	Lactococcus lactis	
	Broth Culture	Slant Culture
Gram stain		
Morphology		
Arrangement		

Questions

1. Did growth occur at different levels in the agar deep? _____

2. Were any of the bacteria growing in the semisolid agar deeps motile? _____ Explain.

3. What other methods can be used to determine motility? _____

_____

4. What is the primary use of slants? _____

Of deeps? _____

Of broths? _____

5. Can you determine whether a broth culture is pure (all one species) by visually inspecting it without a microscope? _____ An agar deep culture? _____

An agar slant culture? _____

6. When is a loop preferable for transferring bacteria? Use an illustration from your results to answer. When is a needle preferable? _____

_____

_____

7. What is the purpose of flaming the loop before use? After use? _____

_____

_____

8. Why must the loop be cool before touching it to a culture? Should you set it down to let it cool? How do you determine when it is cool? _____

_____

_____

9. Why is aseptic technique important? _____

_____

_____

Critical Thinking

1. Why was the arrangement of *Lactococcus* from the broth culture different than that from the slant culture in the second period?

2. What evolutionary advantage would there be to the formation of a pellicle in a liquid medium by a bacterium?

3. How can you tell that the media provided for this exercise were sterile?