

## **BASIC BACTERIOLOGICAL TECHNIQUES**

An experienced microbiologist employs many techniques and skills when handling microorganisms. More so than in any other field of science, the skills of a microbiologist need to be practiced and refined to achieve a level of proficiency. Microscopy is one skill you are already learning and will soon master with further practice.

Other skills of the microbiologist allow for the safe handling and manipulation of microorganisms. Isolation and investigation of microorganisms are formidable tasks confronting both novice and experienced microbiologists. The skills employed to achieve these objectives are called **aseptic technique**. Aseptic technique is necessary to prevent contamination of **Pure culture** (cultures containing a single species) used in the laboratory. Bacteria and fungi are ubiquitous in the environment and are potential contaminants during most microbiological manipulations. Clearly, a culture contaminated with unwanted organisms will yield spurious results in procedures designed to study the original organism.

Aseptic technique also reduces the risk that people may be exposed to pathogens studied in the laboratory. The risk of exposure extends to persons who contact surfaces, instruments or wastes contaminated with microorganisms. For example, many people could be exposed to a pathogen unwittingly carried from the lab on contaminated clothing. Thus, you should strive to master the aseptic techniques by which you will control the spread of microorganisms studied this semester.

### **Summary of exercise**

- I. You will practice making aseptic transfers between culture media.
- II. You will practice making a streak plate using a provided mixed bacteria culture
- III. You will inoculate a plate of nutrient medium from an environmental site to demonstrate the prevalence of microorganisms in the environment and use culture to isolate an unknown bacterial species.
- IV. You will examine bacteria cultured on solid media to study the importance of colony morphology in identifying bacterial types.

## I. Practice Aseptic Transfers

The technique that you will use most frequently during the semester is aseptic transfer of bacteria. You will need to transfer bacteria aseptically whenever you inoculate culture media with an organism. The **inoculating loop** is most commonly used to transfer cells to or from broth and solid culture media. An important point to remember that seeing a mass of bacteria on the loop during a transfer is not necessary (although it is reassuring)—even a single cell is adequate to start a new culture. Probably the most important rule for any aseptic transfer is to **sterilize the inoculating loop BEFORE and AFTER each transfer**. Why?

The techniques for doing aseptic transfers will be demonstrated in class. The manner in which you hold the tubes, caps and inoculating loop are important. You are encouraged to ask for assistance or a critique while you perform the transfers.

### Supplies

4 TSA slants (red caps)  
*Escherichia coli* broth culture (brown cap)  
plated cultures *Staphylococcus aureus* (to be shared with other teams)

### Procedures

Each student in a team should:

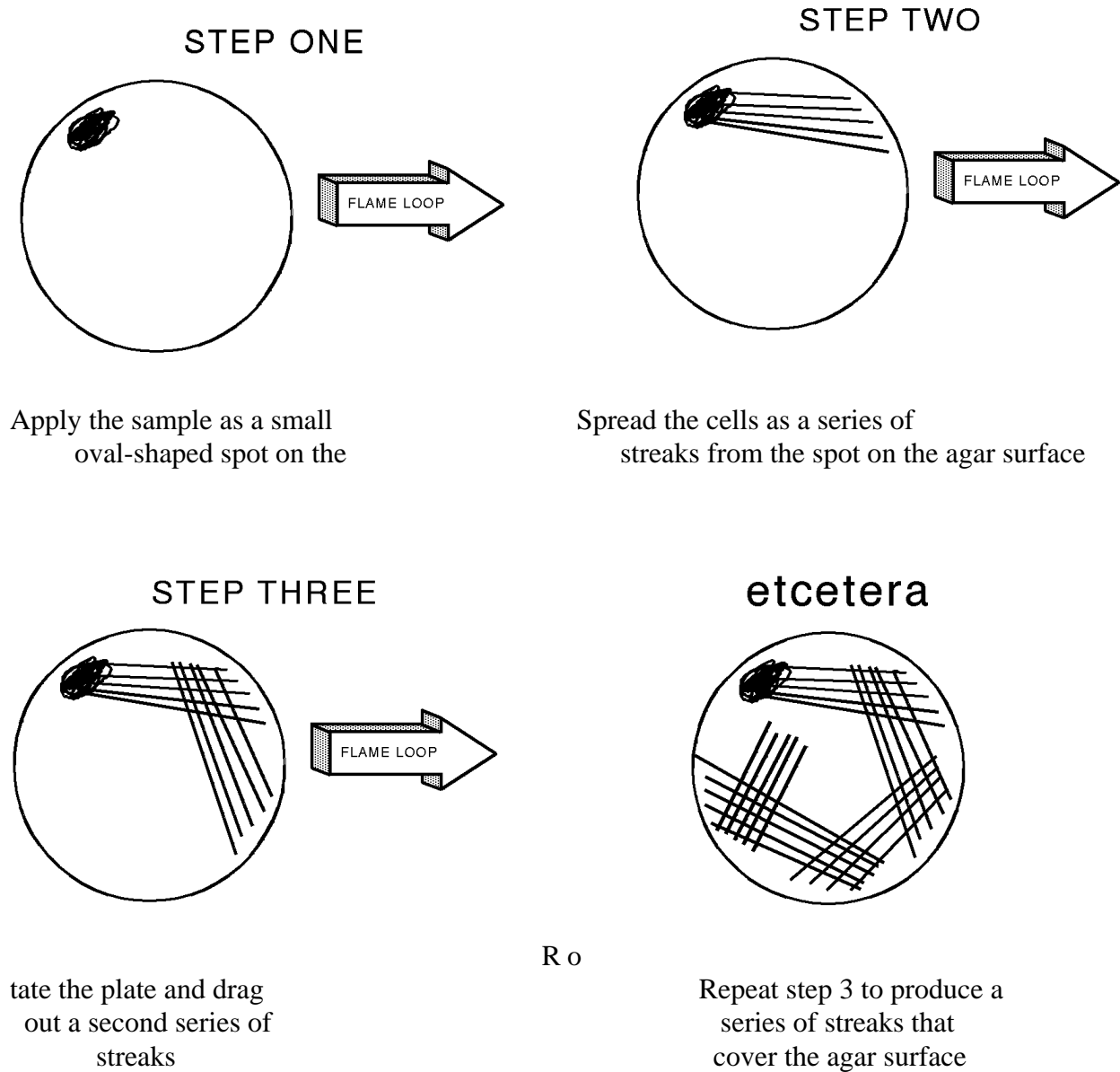
1. Transfer *Escherichia coli* from a broth culture to a Tryptic Soy Agar (TSA) slant.
2. Transfer *Staphylococcus aureus* from a plate to an agar slant.
3. Incubate all of the inoculated cultures at 37°C for 24- 48 hours, and then observe whether the transfers were successful, and then discard the cultures.

## II. Learning the streak plate technique

Streak plating is a powerful technique that microbiologists employ for a number of purposes, including 1) evaluating the purity a bacterial culture, 2) examining the diversity of species in a sample, 3) separating a species from a mixed culture so that a pure culture can be prepared, and 4) studying the colony characteristics of a species. The streaking technique will be demonstrated during the lab period; however, the general procedure and strategy is described below.

The primary objective of streak plating is to obtain **isolated colonies**. Remember, a "colony" is bacterial growth on solid medium that originated from a single cell. To obtain isolated colonies, individual cells must be dispersed over the surface of the agar medium. This is accomplished by spreading the sample as a series of "streaks" on solid medium in a petri plate, as shown in Figure 1. The pattern of streaking shown below consistently yields good results in this course, but is not the only technique that can be used. Note that the inoculating loop is **flame sterilized between each step**, and with each series of streaks the density of cells is decreased.

**Figure 1. Performing a streak plate**



On a correctly performed streak plate, the colony growth should appear as shown in Figure 2.

## Supplies

2 plates of TSA medium  
mixed bacteria culture (green cap)

## Procedures

### Helpful hints for streak plating:

1. Merely lay the loop on the medium when streaking. Do not dig the loop into the medium—you are not planting corn.
2. Be careful to cross only the previous series of streaks. It is imperative that the last series of streaks does not run into the original spot of inoculation. Why?
3. Do not let the streak plate incubate too long. Rapidly expanding colonies isolated after 24 hours may merge with neighboring colonies after 48 hours.

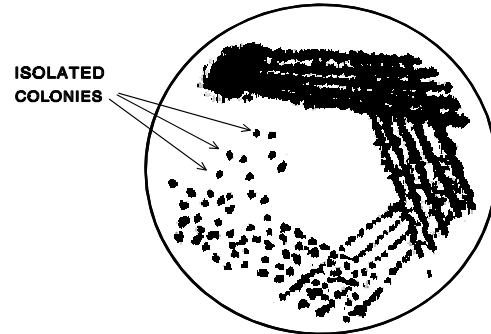


Figure 2. Incubated Streak Plate

### **Each student should preform a practice streak plate.**

1. From the mixed culture (green cap) prepare a streak plate using your inoculating loop.
2. Show your plate to the instructor for feedback on your technique.
3. Incubate the plate at 37°C for 24 - 48 hrs.
4. Observe the quality and results of your technique. Did you obtain isolated colonies of two types?
5. Compare the characteristics of the colonies to those studied in Part IV to identify the two species in the mixed culture; Record these in the space provided below Table 1.

## **III. Isolating a pure culture of an unknown bacterium.**

### **There are two parts to this exercise:**

- 1) During this lab period you and you lab partner will each swab a TSA plate from two different environmental sources.
- 2) Later during the week, your team will select a single colony from one of the plates inoculated in part 1, and perform two sequential streak plates for bacteria isolated from one of these plates (see Figure 3). You will then transfer bacteria from an isolated colony on the second plate to a slant tube of TSA. This will be the “Semester Unknown that your group will maintain and characterize throughout the semester.

## Supplies

- 6 plates of TSA medium
  - 2 for use today
  - 4 for isolation your semester unknown
- sterile cotton swabs
- tube of sterile water (black cap)
- 1 TSA slant (screw cap tubes, in refrigerator)

### Part 1.

1. Each member of a team should inoculate a separate plate, and a few suggestions for exposing the plates are given below. Feel free to use your imagination, but to minimize the possibility of isolating pathogens, avoid body orifices. Each student can choose a different site, but remember, your group will isolate only a single unknown.

- Obtain a tube of sterile water and a sterile cotton swab. Dampen the cotton swab in the water, then wipe the swab over a surface to be sampled. Finally, fully swab the surface of the petri plate, and recover. Feel free to sample sites inside or outside.
- Inoculate from soil, or from Goose Run
- Swab some money
- Use your imagination (but don't be gross)
- Hint: avoid bathrooms – these are actually cleaned with disinfectant.

2. Incubate the plate in the 37°C incubator for 24 - 48 hrs.

### Part 2. Begin isolation of your semester bacterial unknown (see Figure 3)

**These steps will be done when you return to the lab. Remember, your group will isolate only a single type of bacterium to serve as your semester unknown. You will both perform streak plates to gain experience.**

#### First streak plates

1. The team should select a colony from one of the plates inoculated in Part 1.
2. Each student should prepare a streak plate for bacteria obtained from that colony.
3. Incubate the plate at 37°C for 24 - 48 hrs.
  - ★★★ If you do not get isolated colonies on either of these plates, discuss the results with the instructor.★★★

#### Second streak plates:

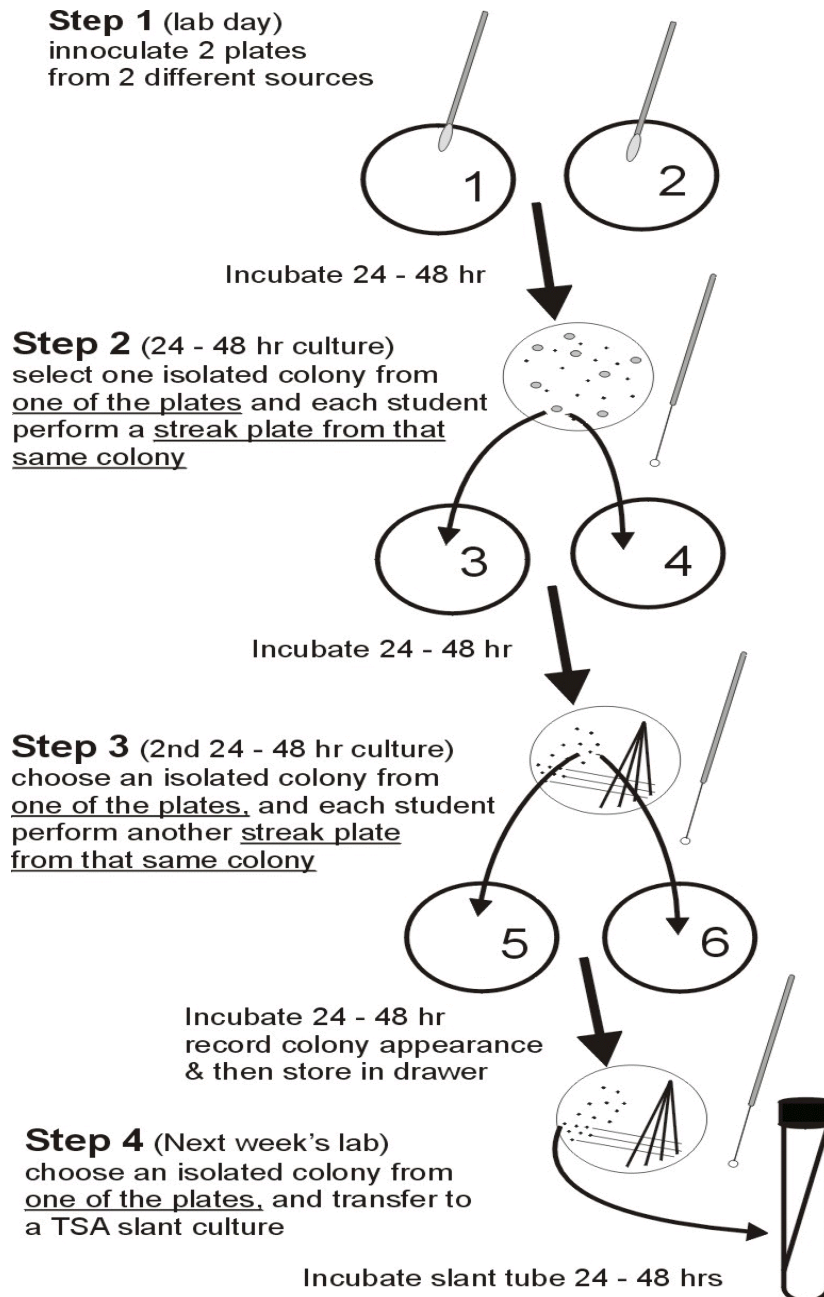
1. Select an isolated colony from one of the plates, and each student should restreak the bacteria onto another plate. Each student should use the SAME colony to prepare the streak plate.
2. Incubate the plate at 37°C for 24 - 48 hrs.
  - ★★★ If you do not get isolated colonies on either of these plates, discuss the results with the instructor.★★★
3. In Table 2 record the characteristics of an isolated colony of the type that you will use to prepare your pure culture.
4. **Store the plate in your drawer until next week – do not leave it in the incubator.**

**Preparing the pure culture (during next week's lab):**

1. Transfer growth from an isolated colony of the type you described to a slant tube.
2. Incubate the plate at 37°C for 24 - 48 hrs.
3. Transfer the culture to your drawer for storage.

During the Cytology Lab period you will test the purity of your culture, and begin a semester-long characterization of the bacteria.

**Figure 3. Procedure for Isolating Semester Unknown**



## IV. Characteristics of colonial morphology

When a clinical microbiologist begins identification of a potential pathogen, a sample from the patient is streaked on solid medium and the types and appearances of bacterial colonies are carefully inspected. The appearance of a bacterial colony is the first important clue in the identification of bacteria. Bacterial species produce colonies with many unique and striking features, although the specific characteristics of the colony will depend upon the type medium on which the cells are grown. Many features of bacterial colonies can be used in identification, and some of these are summarized below and on the next page. Colonies differ markedly in **pigmentation**; colonies may be colored, transparent, translucent or opaque. Some bacteria produce pigments that diffuse into the surrounding medium. The physical appearance of bacterial colonies is, however, influenced by a number of other factors. These include the length and temperature of incubation, the type of medium upon which the bacteria are cultured, the density of cells on the plate, and even the particular genetic strain of a bacterial species. These conditions should be specified when describing colony appearance.

### Objectives














The goal of this exercise is for you to learn to distinguish the great variety of colonial forms, and to use this information to help in the identification of your unknown. You should also learn to distinguish colonies of bacteria from those of fungi. The colonies of common molds frequently (but not always) have a very "fuzzy" surface raised well above the medium surface.

### Procedure

1. From the selection of bacteria (not fungi) provided on plates, identify a species that demonstrates each colony trait listed in Table 1.
2. Examine the demonstration plates of fungi. What are the characteristics of fungi growth that distinguishes it from bacterial colonies?
3. Examine the appearance of the colony of your unknown after streaking but before you transfer it to the TSA slant. Record your observations in Table 2.

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These are some terms used to describe the characteristics of bacterial colonies. Also available in lab are photographs that illustrate some of these characteristics.

Margin (edge)	Elevation	Surface texture	Colony shape
<b>Entire (smooth)</b> 	<b>Sunken</b>  (Below medium surface)	<b>Glossy</b> Shiny and reflective Glistening	<b>Circular</b> 
<b>Undulate (wavy)</b> 	<b>Flat</b> 	<b>Dull</b> Smooth surface but not glossy; non-reflective	<b>Irregular</b> 
<b>Lobate</b> 	<b>Convex</b> 	<b>Granular</b> Roughened surface; possibly powdery in appearance	<b>Spreading</b> rapidly spreads over plate
<b>Lobed</b> 	<b>Umbonate</b> 	<b>Contoured</b> Uneven surface, with undulations and crevasses	<b>Punctiform</b> Forms numerous very small 'pin-point' colonies
<b>Rhizoid</b> 	<b>Pulvinate</b> 	<b>Wrinkled</b> Folded appearance; like a dried raisin	<b>Mucoid</b> Glossy, but sticky and gooey to the touch
<b>Filamentous</b> 	<b>Other considerations:</b> Not all colonies fit neatly into specific categories; often other descriptions are needed to fully describe the appearance. Also, colony appearance can be influenced by growth conditions, including the type of medium on which the cells are grown.		

**Pigmentation:**

1. Color may be: white → milky → creamy → to various distinct colors
2. Opacity may be: transparent → translucent → opaque
3. Color may be: Uniform, Uneven or Patterned, Changing over time

Name: \_\_\_\_\_

**Turn in one set of results per group. Results must be neatly prepared. Sloppy work will lead to a grade deduction.**

**Table 1. Examine the colonies of the bacteria on display.** Select five species and write descriptions for each trait (use proper terminology where appropriate., and write entire name of organism with correct spelling).

Species	Margin	Elevation	Surface	Shape	Color

Based upon colony characteristics, which species were in the mixed culture?

\_\_\_\_\_ and \_\_\_\_\_

### Fungal colonies

Describe the appearance of two fungal species on display. (The terms used to describe bacterial colonies may not be relevant to fungal colonies.)

1 \_\_\_\_\_:

2. \_\_\_\_\_:

In general, how can colonies of fungi be most easily distinguished from those of bacteria?

**Table 2. Identify the colony characteristics of your unknown.** Also copy these descriptions onto the summary sheet. Also describe any other special characteristics of the appearance of your unknown

Margin \_\_\_\_\_

Elevation \_\_\_\_\_

Surface \_\_\_\_\_

Shape \_\_\_\_\_

Pigmentation \_\_\_\_\_

Other:

**Copy these results and others for your semester unknown to the Semester Unknown Summary forms.**

**Also turn in an answer to these questions. Lab partners should discuss and draft an answer, but only one typed answer should be turned in.**

A Discuss the reasons for performing a streak plate?

B. Suppose you prepare a streak plate and after 48 hours in the incubator you do not find isolated colonies –the bacterial growth merely looks like solid lines drawn across the plate. What are four changes in your streaking technique that you might attempt next time to improve the results?