

MCI Mixed Culture Isolation

Learning Objectives

The student will

- Use aseptic techniques in the safe inoculation of various forms of media.
- Follow oral and written instructions and manage time in the lab efficiently.
- Apply correct terminology regarding microbiological techniques, instruments, microbial growth, biochemical testing, media types and forms and epidemiology when making observations.
- Correctly perform various inoculation techniques including the quadrant streak and the T streak techniques and describe each technique's purpose.

Background/Theory

You and your partner each sampled a mixed culture containing *Micrococcus luteus* and *Staphylococcus epidermidis* in a previous exercise. Recall that the mixed broth culture was a light brown color, identical to all the other pure culture broths you had. Not only is it impossible to identify the organism by the appearance of the broth, one cannot tell whether or not a broth contains only one organism or several. Streaking a broth culture onto an agar plate using an isolation streak, on the other hand, will allow you to begin to visualize the different microbes in the mixture based on the differences in appearance of the colonies. Your plate from the mixed culture T-streak, called a **primary streak plate**, likely shows two types of growth, yellow *M. luteus* colonies and white *S. epidermidis* colonies.

Thus far, we have imagined a single cell dividing and growing up into a single colony. In reality, this rarely occurs. Many bacterial cells stick together in doublets, tetrads (groups of 4), small clusters or short chains. When you spread them out on a plate, these small groupings can remain intact. In many cases it is the small grouping, rather than a single cell, that grows into a single colony. We call these groupings **colony forming units, CFUs**. Keep in mind the difference between a colony and a colony forming unit. A **colony** is a mass of cells visible to the unaided eye. A **CFU** is a microscopic single cell or small group of cells. Because a CFU is defined as a grouping from which a colony forms, it is theoretical. Even when you visualize cells under the microscope you will not see CFUs. The concept of a CFU is a past entity. By definition, the only way you have a CFU is if a colony has formed from it.

If it arises from a single CFU, a colony is composed of one type of cell; it is **pure**. Consider a *Micrococcus luteus* colony on your mixed culture plate. Among all those yellow *M. luteus* cells, is it possible that there are a few *S. epidermidis* cells lurking? The colony may look pure, but one cannot be so sure. To ensure a pure culture, you can sample a given colony and streak it onto a new plate called the **secondary streak plate**. If the secondary streak plate shows one type of colony, your confidence in the purity of your culture is increased.

One way that microbiologists can purify or isolate individual organisms from a mixed culture is by using a series of streak plates. The following factors increase the likelihood that the resulting culture is pure.

- You used good aseptic technique.
- The secondary plate was struck from an isolated colony on the primary plate and the original colony looked pure.
- The secondary plate shows isolated colonies and all of them look the same (same color, relative size, texture etc.)
- There is no sign of contamination on the plate.

If the secondary plate does not meet one of the criteria above, you can make a **tertiary streak** by following the same steps. You can make successive streak plates until you have a pure culture. The entire process of taking a mixed culture and producing pure cultures from it is called **isolation**. Over the next couple of weeks, you and your partner will isolate the 2 organisms from one of your mixed culture plates.

Experiment/Exercise

Materials

One TSA or NA plate per person per week

Cultures

Mixed culture t-streak plate *Micrococcus luteus* & *Staphylococcus epidermidis*

Procedure Lab 1

1. Examine your and your partner's t-streak of *Micrococcus luteus* & *Staphylococcus epidermidis* mixed culture from the SI exercise.
2. Pick the one plate that shows the best separation of the two organisms. Look for the plate that shows both types of growth, white *S. epidermidis* and yellow *M. luteus*. You may want to consult with your instructor to choose the best plate. Dispose of the other plate.
3. From this one plate, you and your partner will make secondary T-streak plates from each of the colony types. You sample an isolated colony of *M. luteus*, the convex yellow colonies. Your partner will sample an isolated colony of *S. epidermidis*, the white colonies. Label each plate appropriately (Use only your name on your plate. Use only one organism name on your plate.) These plates are called secondary streak plates.
4. If only one type of colony is present, consult your instructor. You may need to sample an area of confluent growth.
5. Put the primary streak plate in the bag for storage. If your secondary plate does not grow, you will have the primary plate to try again.
6. Even though you begin with the same plate, you and your partner will each have your own secondary streak plate. Be sure the label has only your name and your organism.
7. Place your plate for incubation. Remember, plates are always incubated upside-down.

Procedure Lab 2

1. Look at your secondary streak plate from last week. Evaluate your streaking technique in terms of the criteria listed in the SI exercise. Record your observations.
2. Evaluate the purity of your culture using the criteria stated above.
3. If you were forced to sample an area of confluent growth last week, your plate will likely show more than one type of colony this week. This is OK. Isolating a pure culture from a mixed culture is a process. Sometimes it takes several generations of streak plates to get there. Hopefully, you will see some separation on this secondary streak plate. You may need to consult your instructor for direction on what to sample for the next plate.
4. Even if all of the conditions above are true, your culture may still have some contaminating cells "hidden" among the predominant growth. To achieve greater certainty of purity, you will make at least one more t-streak plate from a colony of the organism you are trying to isolate. (Your partner will have a different organism.)
5. From one isolated colony make a 3^o T-streak plate. Label the plate appropriately. (Use only your name on your plate. Use only one organism name on your plate.)

6. Set your 3^o plate in the space designated for incubation. Put your 2^o plate in the bag for storage along with your 1^o plate. Do not dispose of it yet.

Procedure Lab 3

1. Look at your 3^o streak plate from last week. Evaluate your streaking technique in terms of the criteria listed on the SI exercise. Record your observations.
2. Evaluate the purity of your culture using the criteria on the first page of this exercise.
3. If your culture does not appear pure at this point, consult with your instructor. If you were forced to sample an area of confluent growth last week, your plate will likely show more than one type of colony. You may be allowed one more streak plate.
4. Compare your 3^o plate and your 2^o plate. Choose the one you think best demonstrates the T-streak technique and place it in the location designated for plates to be graded. Dispose of the other plates properly.

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Lab Report: Mixed Culture Isolation

Name _____

Lab Section _____

Data and Observations

2° Plate Technique	2° Plate Purity (written comments)
<input type="checkbox"/> isolated colonies at least three <input type="checkbox"/> streak pattern: correct order, correct direction <input type="checkbox"/> amount crossovers (not too little, not too much) <input type="checkbox"/> entire surface used <input type="checkbox"/> no gouging of the agar <input type="checkbox"/> no inappropriate crossovers <input type="checkbox"/> 90° angle of streaks <input type="checkbox"/> adequate room for final area <input type="checkbox"/> growth pattern: a lot to a little <input type="checkbox"/> label : all components, legible, on bottom of plate incubated upside-down	
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Post Lab Questions

1. A mass of cells, visible to the naked eye that has grown from a single cell or a small chain, pair or small cluster of cells is a _____.

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2. A _____ is the original single cell or a small group (short chain, pair or small cluster) of cells from which a colony forms. This is microscopic and, thus, NOT visible to the unaided eye.
3. One can separate CFUs on the surface of the agar sufficiently to produce isolated colonies upon incubation using one of several _____ methods.
4. The _____ or the _____ technique can be used when the sample is of high density.
5. The _____ technique is used when the sample is of relatively low density.
6. This plate shows signs of two different organisms growing. In other words, it is contaminated.
How do you know?

