Using Sterile Technique to Inoculate Bacterial Plates

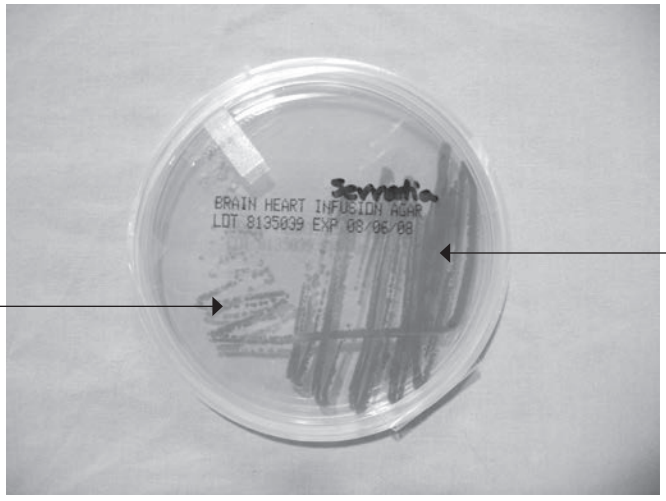
A sterile, or aseptic, technique is used to prevent microbial organisms from contaminating any surface other than the specific location where they are being transferred or encouraged to grow. To keep bacteria from growing where they are not wanted (surfaces such as your hands, desk, or media plates where they were not intended), the bacteria–and anything that touches the bacteria– must not touch any additional surfaces. To grow a particular bacterial strain on a specified media plate, you will use a disposable or flame-resistant inoculating loop (these instructions will cover the use of a disposable plastic inoculating loop).

Read all the steps below, and study the pictures carefully. Perform a "dry run" of this technique with a partner while he or she watches and points out improvements. When you are ready, follow the steps below, paying very careful attention not to touch any surfaces with the inoculating loop, the bacterial tube, or the cap of the bacterial tube.

If you are to keep the media plates, culture tubes, and inoculating loops free of any additional unwanted organisms, it is important that they remain closed at all times, except when the bacteria are being transferred. The photos in this series are taken with ungloved hands so the students can get a clear view of the position of the fingers. All students should wear gloves and should wash their hands and fingernails with soap and water after the procedure.

1. Arrange the media plates, bacteria source, inoculating loops, and trash receptacle within easy reach. Do not open the inoculating loops, culture tubes, or media plates. Label the media plates with (a) the date, (b) the bacterial strain, and (c) the medium name before beginning.
2. The inoculating loops should remain in the sterile packaging. Touch them only at the center of the handle with gloved hands. **Never** touch either end to anything other than the bacterial culture tube or a media plate. Read the rest of this procedure so you understand what you will do with the inoculating loop.

   One end of the inoculating loop will be used to dip into the bacteria growing in the culture tube. This end will be used to make a zigzag pattern (six to eight times back and forth) on half of the media plate. The inoculating loop can then be turned over so the other end can be used to slash across the zigzag one time and to make a second zigzag pattern across the remaining half of the media plate.



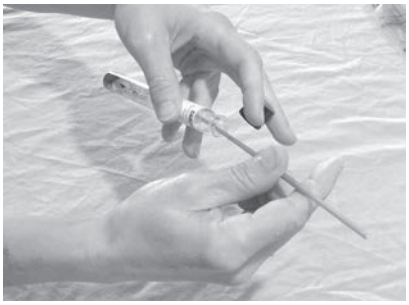
First zigzag streak of the inoculating loop

Second zigzag streak using the opposite end of the inoculating loop

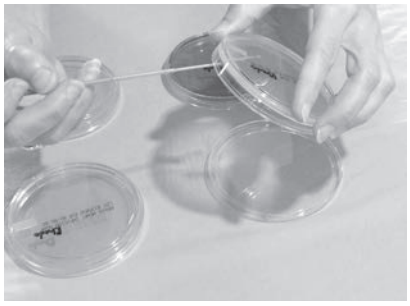
Using Sterile Technique to Inoculate Bacterial Plates (continued)

This technique will smear the bacteria from the culture out across the medium in a thin film during the first streak, and then the second streak will spread a small number of bacteria away from the others to allow the growth of individual colonies.

3. The bacterial culture tubes should be opened only briefly by lifting the cap just enough to slide the inoculating loop inside without touching the loop to the cap. The cap should never be set down in the process, and the tube should be sealed immediately after the loop is removed. You may do this procedure using two people, or you can practice the technique of unscrewing the top and holding the cap and tube in one hand while you work with the inoculating loop.



4. The media inside the plates should never be touched by anything except the inoculating loop as it adds the bacteria. The covers to the media plates should always remain closed and should be lifted only enough to allow the inoculating loop to spread the bacteria. When the lid is lifted, it should remain facing down and should hover over the bottom of the plate to minimize the chance of contaminating fungal or bacterial particles entering. The lid should never be turned over or set on the table and should be closed immediate after inoculation.



5. After a single plate has been inoculated, the inoculating loop should go directly into a trash receptacle. A new loop will be needed to inoculate the next media plate using the same technique. After all the plates have been inoculated, the plates should be taped shut using Parafilm® and plastic wrap or tape. The *Rhodospirillum rubrum* bacterial plates will need to be taped so they are airtight in order to encourage the bacteria to grow anaerobically. None of the media plates should be opened for any reason after this point in the procedure.

Growing Bacterial Strains on Various Media—Lab Experiment

Introduction

Provide the information requested below.

What is the question that you are asking in this experiment?

Give two possible answers to the question above (these are your hypotheses).

1._____

2._____

The null hypothesis for this experiment is this:, Different strains of bacteria will grow equally well on various types of nutrient media.

After we have seen the results of this experiment, we will either accept or reject the null hypothesis. At this point (before you have seen any experimental results), do you think the null hypothesis will be accepted or rejected?

_____

Using three examples, explain how this lab experiment could help a scientist use bacteria in biotechnology.

_____

_____

_____

Methods

Fill in the information requested below.

Materials–List the materials you need to perform this experiment.

_____

_____

Procedure–Make a quick sketch of each step in this procedure:

Setting up:

Spreading the bacteria on the plates:

Cleaning up:

Growing Bacterial Strains on Various Media—Lab Experiment (continued)

Results

Fill in the data chart with a plus (+) symbol if the bacteria grew or a minus (–) symbol if the bacteria did not grow. Record observations about your results in the space below. Include color, thickness of colonies, and any other details.

Data chart:

Name of Bacterial Strain	Lab Group	Nutrient Agar	Brain Heart Agar	Tryptic Soy Agar	MacConkey Agar
Escherichia coli					
Rhodospirillum rubrum					
Pseudomonas fluorescens					
Enterococcus faecalis					
Branhamella catarrhalis					
Serratia marcescens D1					

Observations on the plated media:

Observation on the oil-consuming bacteria:

Conclusion

Answer the following reflection questions.

Explain why some bacterial strains grew on certain nutrient media but did not grow on other nutrient media.

Which strains of bacteria were able to grow on the most types of media?

Which bacterial strains appeared to have more specific nutrition or environment needs?

Growing Bacterial Strains on Various Media—Lab Experiment (continued)

Explain how positive controls were used in this experiment.

Revisit your hypotheses in the Introduction section. Explain which predictions were true, and back up your explanations with data from the experiment.

Explain which parts of your hypotheses were not quite accurate, and back up your explanations with data from the experiment.

Now that you have seen the results of this experiment, would you accept or reject the null hypothesis?

What types of errors occurred or could have occurred to alter the results?

If you were to perform another experiment using the same materials, what question would you test?

What does the variation in growth of these bacteria suggest about the growth habits of bacteria that occur naturally in soil or water of an ecosystem?

If you were growing a bacterial strain in the lab on one particular type of nutrient agar and you found that those bacteria could consume plastic grocery bags as a carbon energy source, what are some ways that you could encourage those bacteria to help clean up a landfill?

Oil-Consuming Bacteria—Lab Experiment

Introduction

Oil-consuming bacteria have a number of interesting applications such as detoxifying landfill waste, cleaning up oil spills, or decontaminating groundwater. To better sell these specialized organisms to companies that need them, you would need to quantify how much of a particular substance the bacteria can consume over a given time. Design an experiment that addresses the following question:

How much oil does this bacterial strain consume in a given time?

Methods

List the materials you will need for this experiment:

_____

_____

_____

_____

Write out the steps of your lab procedure next, and then draw the steps of the procedure on a large piece of poster board to present the experiment to the class.

Procedure:

1._____

2._____

3._____

4._____

5._____

6._____

7._____

8._____

9._____

Oil-Consuming Bacteria—Lab Experiment (continued)

What are the variables in this experiment?

What are the controls?

Results

Draw a data table that would be used to collect the information you expect to get from your procedure.