

## Separation of Microorganisms from Mixed Culture---Streak Plate

As you may remember from your first laboratory, bacteria exist in the environment as mixed populations. In order to study the morphological, physiological and biochemical characteristics of a single species, we must first isolate it from all other organisms and grow it in pure culture.

Over the next week or so, you will practice the streak plate method for the isolation of microorganisms. You can practice several different methods of this procedure shown on p. 79 of your lab manual or you can use the method demonstrated in the lab. Your goal for this exercise is to separate the microorganisms from a mixed culture into well separated colonies that could be plucked from the plate with an inoculating loop or needle in such a way that your won't touch a neighboring colony. For a picture showing a well-isolated streak plate, refer to Fig. 19.1 on p. 76 of your lab manual.

**Exercise 1--Day 1:** For this exercise, you will be given several different mixtures of bacteria. One broth tube contains three microorganisms (Tube # 1). These organisms are *Escherichia coli*, *Micrococcus luteus* and *Serratia marcescens*. You should be able to separate these three microbes into well isolated colonies by streaking them onto a petri plate of Nutrient Agar. Try streaking two plates. After streaking the plates, incubate one of these plates at room temperature and the other in the incubator. As always, these plates should be incubated in an inverted position.

Record your results.

37°C

R.T. (23°C)

Do you see three different colony types? If so, how are they different?

Are the colonies well separated?

If not, what could you do to aid in the separation process?

**Questions:**

Look up the organisms given in this exercise in the Bergey's Manual and list some of the major characteristics for these organisms in the following table.

| <b>Bacteria</b>            | <b>Shape</b> | <b>Gram Stain</b> | <b>Colony characteristics</b> | <b>Other characteristics of interest</b> |
|----------------------------|--------------|-------------------|-------------------------------|--|
| <i>Escherichia coli</i>    |              |                   |                               |  |
| <i>Micrococcus luteus</i>  |              |                   |                               |  |
| <i>Serratia marcescens</i> |              |                   |                               |  |

Can you determine which colony corresponds to which bacteria? If so, how?

**Exercise 2--Day 2:** Tube #2 contains 2 organisms in the *Staphylococcus* genus (*Staphylococcus aureus* and *Staphylococcus epidermidis*). You should streak these organisms onto a petri plate containing Mannitol Salt Agar. Practice using two plates. Again incubate these plates, inverted, in the incubator and examine them after 24 hours.

Record your results.

Are there different colonies present?

If so, in what way are they different?

Are the colonies well separated?

If not, what could you do to aid in the separation process?

**Questions:**

Look up the organisms given in this exercise in the Bergey's Manual and list some of the major characteristics for these organisms in the following table.

| <b>Bacteria</b>                   | <b>Shape</b> | <b>Gram Stain</b> | <b>Colony characteristics</b> | <b>Mannitol fermentation</b> |
|-----------------------------------|--------------|-------------------|-------------------------------|------------------------------|
| <i>Staphylococcus aureus</i>      |              |                   |                               |                              |
| <i>Staphylococcus epidermidis</i> |              |                   |                               |                              |

**Media Types**

Your text book in Chapter 3, discusses 4 general types of media based on their physical properties, solid, semi-solid and liquid media types. We've used solid and liquid media types so far in this laboratory. Identify types of media found in these categories.

Solid:

Semi-solid:

Liquid:

Non-liquifiable solid:

More importantly, however, are media types that allow microbiologists to isolate bacteria, maintain stock cultures, and determine biochemical characteristics. Define the following types of media and provide an example.

General purpose:

Enriched:

Selective:

Differential:

**Exercise 3--Day 3:** To emphasize both selective and differential media, streak Tube #3, containing *Escherichia coli* and *Enterobacter cloacae* onto a plate of Levine EMB. Practice using two plates. Again incubate these plates, inverted, in the incubator and examine them after 24 hours.

Record your results.

Are there different colonies present?

If so, in what way are they different?

Are the colonies well separated?

If not, what could you do to aid in the separation process?

Fill in the following table using Bergey's Manual.

| <b>Bacteria</b>             | <b>Shape</b> | <b>Gram stain</b> | <b>Colony characteristics</b> | <b>Misc. Characteristics</b> |
|-----------------------------|--------------|-------------------|-------------------------------|------------------------------|
| <i>Escherichia coli</i>     |              |                   |                               |                              |
| <i>Enterobacter cloacae</i> |              |                   |                               |                              |

The bacteria, *Escherichia coli* and *Enterobacter cloacae* belong to a category called coliforms. Define what a coliform is.

Levine EMB is both a selective and differential media. It selects for Gram negative bacteria. Use both your text book, the Difco Manual or the Microbiological Media book to determine how Levine EMB is differential. Hint: in differentiates between coliforms.

Mannitol Salt Agar used in Exercise 2 is also a selective and differential media. Use various sources to determine how Mannitol Salt Agar is selective and how it is differential. Hint: what does the salt do in the agar and what does the mannitol do?

Your lab manual discusses a second method of microbe separation called the Pour Plate method. How is this method different?

Which in your opinion is the best method? Explain.

**Sources:**

1. Benson, Microbiological Applications: Exercise 18.
2. Bergey's Manual of Determinative Bacteriology.
3. Difco Manual.
4. Microbiological Media Handbook.
5. Talaro, Foundations in Microbiology, Chapter 3.