

Guide to Plating Bacteria Using Sterile Technique: Teacher Instructions

By Rebecca Milholland, University of Arizona, Department of Pharmacology/Toxicology

Supplies:

Wire loops (1 for each student)

Bacteria Media Plates

Bacteria of Choice (*Serratia marscecens*, *Eschericia coli*)

Recipes:

Bacteria Media Plates

Mix the following ingredients with 1L (20ml media per plate, makes about 35 plates) of distilled water:

10g NaCl

10g tryptone

5g yeast extract

20g agar

Autoclave the solution (121 ° C, 30 minutes); wait for the solution to cool (should be cool enough to handle but not cold) and pour carefully into the Petri dishes avoiding bubbles as much as possible. Allow to gel or solidify at room temperature overnight (O/N). LB-Plates can be stored in a refrigerator for up to 2 months or at room temperature for a couple of days.

Procedure:

- A.** Have Students obtain one Bacteria Culture Plate and a wire loop. The Culture Plates contain media (food) for the bacteria in a gel or Jell-O type form.
- B.** Have the students label the bottom of the Bacteria Culture Plate with:
 - 1) Their Name
 - 2) Today's Date
- C.** Have the students move the Bacteria Culture Plate and wire loop near a burning Bunsen burner.
- D.** Have the students flame the wire loop by placing the end of the loop in the flame until it turns red hot. This procedure acts to sterilize the end.

- E. After the wire loop has been flamed, without touching the end to anything, have students wait several seconds for the loop to cool (otherwise you will kill whatever bacteria you are trying to plate by burning them).
- * One way to test whether the loop is cool is to gently touch it to the media in the culture plate. If the loop is still hot, it will make a singing sound when it touches the media.
- F. Once the end of the wire loop has cooled, have students scoop a very small amount of bacteria from the stock culture plate with the loop.
- G. Have the students plate or spread the bacteria onto the media in the Bacteria Culture Plate by using the pattern shown on the next page to plate the bacteria. This pattern will help students get single colonies instead of just a big pile of bacteria all over their plate.
- Students should drag the loop across the media gently being careful not to poke a hole in the media.**
- H. Incubate Plates for 24-48 hr. at 37 ° C.

Student's Guide to Plating Bacteria Using Sterile Technique

Supplies:

Wire loop

Bacteria Media Plate

Bacteria

- A. Obtain one Bacteria Culture Plate and a wire loop. The Culture Plates contain media (food) for the bacteria in a gel or Jell-O type form.
 - B. Label the bottom of the Bacteria Culture Plate with:
 - Your Name
 - Today's Date
 - C. Take the Bacteria Culture Plate and wire loop near a burning Bunsen burner.
 - D. Flame the wire loop by placing the end of the loop in the flame until it turns red hot. This procedure acts to sterilize the end.
 - E. After the wire loop has been flamed, without touching the end to anything, wait several seconds for the loop to cool (otherwise you will kill whatever bacteria you are trying to plate by burning them).
 - F. One way to test whether the loop is cool is to gently touch it to the media in the culture plate. If the loop is still hot, it will make a singing sound when it touches the media.
 - G. Once the end of the wire loop has cooled, scoop a very small amount of bacteria from the stock culture plate with the loop.
 - H. Plate or spread the bacteria onto the media in the Bacteria Culture Plate by using the pattern shown on the next page to plate the bacteria. This pattern will help you to get single colonies instead of just a big pile of bacteria all over your plate.
- * **Drag the loop across the media gently being careful not to poke a hole in the media.**