NAME _____

DATE _____

Introduction to Sterile Technique

In this activity you will set up cultures of a bacterium. In doing so it is important that you follow a set of procedures collectively called sterile technique. Sterile technique prevents contamination of a pure culture by unknown microorganisms. It also protects you and your workspace from contamination by bacteria as you work with the cultures. Even though the bacterium you will work with is a nonpathogen, when grown in great numbers in a culture, almost any bacterium may pose a health risk if mishandled. Fortunately, sterile technique is easy to learn. The basic premise is that all instruments must be sterilized and not touch a nonsterile surface before contacting a culture, and that they be resterilized after they have come into contact with a culture.

Record here the name of the bacterium you will work with. _____

Preparation

Before beginning, wash and dry your hands, put on personal protective equipment as specified by your instructor, and wipe down your workbench with disinfectant.

Place the following in a test tube rack: tube culture of bacteria, nutrient agar slant, and tube of nutrient broth. You will also need a plate of nutrient agar. Label each tube with the name of the bacterium you will inoculate, your initials, and the date. Label the bottom of the plate with the same information. (It is best to label the bottom of a plate since the lids are removable and might be switched.) You will need an inoculating loop and beaker or other holder for the loop.

Setting up your workspace

To prevent reaching across the test tube rack to use the Bunsen burner, position the burner and beaker with inoculating loop on the side of your dominant hand and position the rack on the side of your other hand. For safety, maintain at least 30 cm (12”) between the burner and other objects and between the burner and the edge of your workbench. When it is not in use, the inoculating loop should be in the beaker.

Transferring Bacterial Cultures to Fresh Media (Subculturing)

Bacteria growing in a culture eventually deplete the nutrients and need to be transferred to fresh medium for continued growth.

Observe the bacterial growth in the tube culture. Record its appearance here.

_____

_____

Light the Bunsen burner and adjust the flame to about 7 cm (2¾”) with a visible central blue cone. The hotter parts of the flame are outside this cone, with the hottest part just above the cone.

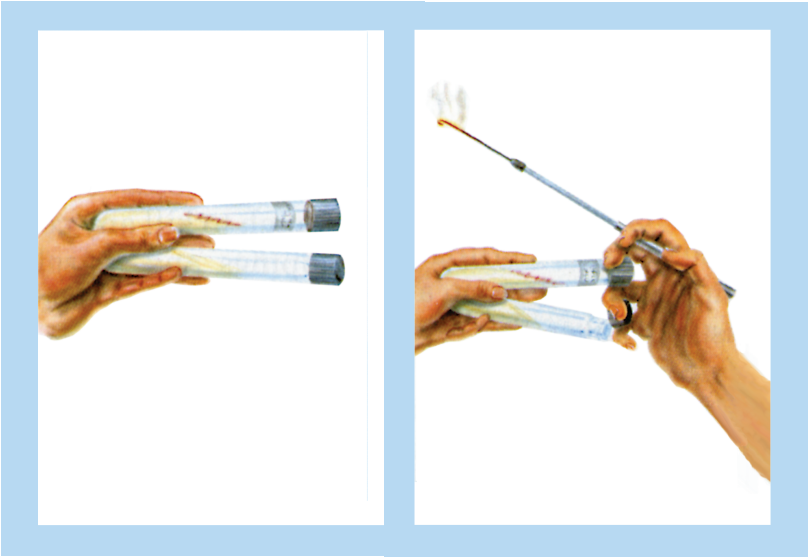
At no point during the following procedures allow the inoculating loop (other than the handle), mouths of the tubes, or inner surfaces of the caps to contact a nonsterile surface (such as your hands).

Transferring to broth

1. Loosen the caps on the bacterial culture and the nutrient broth tube but do not remove the caps.
2. Pick up the bacterial culture tube with your nondominant hand and the loop with your dominant hand.
3. Hold the loop in the burner flame until it glows orange. Do the same for the wire from the loop back to the handle. Allow to cool. Continue to hold the loop as you proceed. (Once you have flamed a loop, do not allow it to touch any surface until you use it to pick up a bacterial sample for transfer.)
4. Grasp the cap of the bacterial culture tube between the little and ring fingers of your dominant hand and remove the cap. Continue to hold the cap as you proceed.
5. Pass the mouth of the bacterial culture tube through the burner flame. This warms the mouth of the tube, creating a rising current of air that will carry any airborne spores up and away from the tube.
6. Insert the loop into the bacterial culture tube and pick up a small sample of culture on the loop. Withdraw the loop from the tube.
7. Flame the mouth of the tube and replace the cap.
8. Return the bacterial culture tube to the rack and pick up the nutrient broth tube.
9. Grasp the cap of the nutrient broth tube between the little and ring fingers of your dominant hand and remove the cap. Continue to hold the cap as you proceed.
10. Pass the mouth of the nutrient broth tube through the burner flame.
11. Insert the loop into the broth and agitate with the loop to loosen bacterial cells and disperse them into the broth. Withdraw the loop from the broth and tap it against the inside of the tube to dislodge any excess broth. Withdraw the loop from the tube.
12. Flame the mouth of the tube and replace the cap.
13. Return the inoculated nutrient broth tube to the rack.
14. Flame the loop as in Step 3 to kill any bacteria that remain on it. Return the loop to the beaker.

Transferring to an agar slant

1. Loosen the caps on the bacterial culture tube and the nutrient agar slant tube but do not remove the caps.
2. Place the bacterial culture tube and the nutrient agar slant tube in the palm of your nondominant hand with the bottoms of the tubes against your palm. The bacterial culture tube should nestle between your index and middle fingers and the nutrient agar slant tube between your middle and little fingers. Use pressure from your thumb to hold the tubes in place.
3. Pick up your inoculating loop with your dominant hand and flame the loop.



4. Grasp the cap of the agar slant tube between the little and ring fingers of your dominant hand and the cap of the bacterial culture tube between your ring and middle fingers and remove the caps. Continue to hold the caps in this manner as you proceed.

5. Pass the mouths of both tubes through the burner flame.
6. Insert the loop into the bacterial culture tube and pick up a small sample of culture on the loop. Withdraw the loop from the tube.
7. Insert the loop into the agar slant tube and, beginning at the lower end of the slant, drag the loop across the agar in a zigzag pattern as you work toward the upper end of the slant. This inoculates as much of the agar's surface with bacteria as possible. Try not to dig into the agar with the loop as you do so.
8. Flame the mouths of the tubes and replace the caps.
9. Flame the loop and return it to the beaker.
10. Return both tubes to the rack.

Transferring to a plate

1. Loosen but do not remove the cap on the bacterial culture.
2. Pick up the bacterial culture tube with your nondominant hand and the loop with your dominant hand. Flame the loop.
3. Grasp the cap of the bacterial culture tube between the little and ring fingers of your dominant hand and remove the cap. Continue to hold the cap as you proceed.
4. Pass the mouth of the bacterial culture tube through the burner flame.
5. Insert the loop into the bacterial culture tube and pick up a small sample of culture on the loop. Withdraw the loop from the tube.
6. Flame the mouth of the tube and replace the cap.
7. Return the bacterial culture tube to the rack.
8. Lift the lid of the petri dish just enough to insert the loop. Never completely uncover the dish. It is best to open the dish clam-shell style. Drag the loop over the agar in a zigzag pattern to cover as much of the agar's surface as possible. Try not to dig into the agar with the loop as you do so.
9. Withdraw the loop and replace the lid.
10. Flame the loop.

Take your new cultures to the area indicated by your lab instructor for incubation. Record here the temperature at which they will be incubated and any other details.

Return your starter culture to where you picked it up, or dispose of it as instructed.

Return all equipment to proper storage. Wipe down your workbench with disinfectant. Wash and dry your hands before leaving the lab.

Observing Your Cultures

Wash and dry your hands, put on personal protective equipment as specified by your instructor, and wipe down your workbench with disinfectant.

Retrieve your cultures and examine them, noting any changes.

Agar slant _____

_____

Broth _____

_____

Plate _____

_____

Do you see any indication of contamination? If so, how might it have happened and how could you prevent it from reoccurring?

_____

_____

_____

_____

Describe a simple procedure that you could use to determine whether or not your broth culture is contaminated.

_____

_____

_____

_____

Return all equipment to proper storage. Return your cultures to storage or dispose of them as directed by your instructor. Wipe down your workbench with disinfectant. Wash and dry your hands before leaving the lab.

Carolina Biological Supply Company
2700 York Road, Burlington, North Carolina 27215
Phone: 800.334.5551 • Fax: 800.222.7112
Technical Support: 800.227.1150 • www.carolina.com
CB163681403