

Aseptic Technique Laboratory

Name:

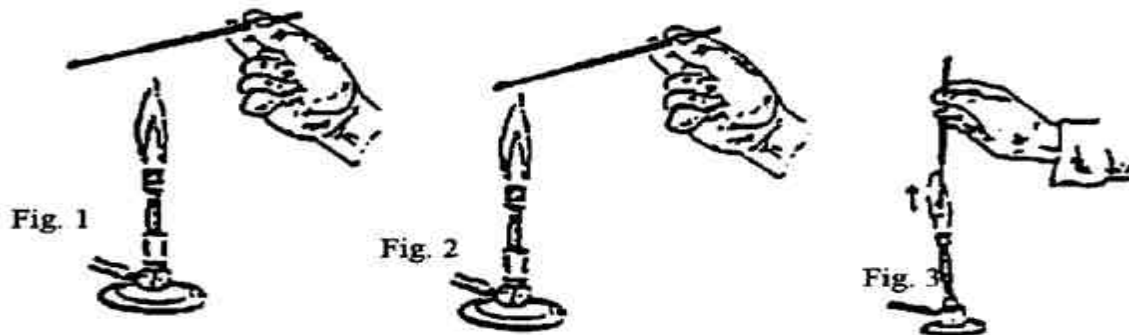
Aseptic techniques will be used in this laboratory as it is used in a clinical laboratory. In the preparation of plating a specimen for isolated colonies, it is necessary to observe the rules of aseptic technique to assure that contaminants are not introduced into a specimen. On a more personal note, adherence to aseptic technique assures that infectious agents are not spread to you, fellow students, or the laboratory surfaces.

The following general rules should be adhered to when working in the microbiology laboratory.

1. The inoculating loop is usually used for making transfers of bacterial cultures. Instructions for the proper technique used to flame a loop with a Bunsen burner are provided on the following page. Allow the loop to cool sufficiently so that any organisms to be tested will not be killed by the hot wire, but do not allow the loop to contact anything during the cooling period or contamination will result.
2. Learn to remove and replace the closures (usually caps) of the test tubes or tops of the Petri dishes with the same hand that holds the loop. The caps must be held during the entire procedure and never placed on the desktop or contamination will result.
3. After the transfer is completed the loop must be sterilized again. Follow the procedure outlined on the following page in Figs. 1-3 to prevent splattering of infectious materials.
4. Always work sitting down.
5. Attention to details and practice will allow you to work both rapidly and accurately.

FLAMING A LOOP

Heat from the **base** of the wire first (Fig. 1) and slowly move towards the loop (tip) (Fig. 2). Heat the wire until it is red-hot (Fig. 3).



LABORATORY CULTIVATION AND ISOLATION OF BACTERIA

Diagnostic bacteriology is concerned with the isolation and identification of bacteria in a specimen from a patient. These specimens, unless from a normally sterile site of the body, rarely contain a single bacterial type, but are mixtures of the disease-producing bacteria and the host's normal flora. Since accurate studies of a bacterial species are possible only through the use of pure cultures, it is necessary to have a reliable and rapid method that will permit the isolation of possible pathogenic organisms. An inoculum from the specimen is streaked on solid agar in a manner, which physically separates most of the bacterial types, permitting them to form discrete colonies.

The method used most often for colony isolation from a clinical specimen or mixed culture utilizes the four-phase streaking pattern described below.

COLONY ISOLATION

Step 1. Using a sterile loop, streak cultures (liquid broth) over one-fourth of the surface of an agar plate. Then flame the loop as described on the preceding page.

Step 2. Air cool a flamed loop or cool it by touching an unstreaked area of agar on the same plate.

Step 3. Pass the cooled loop three or four times over the initial streaked portion of the plate. Streak it, without overlap, to the next quadrant.

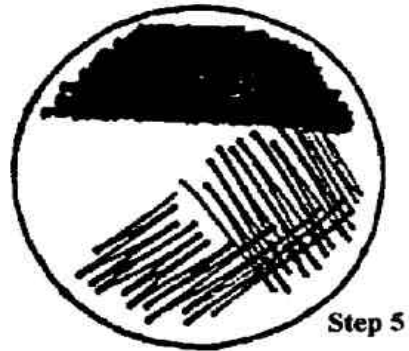
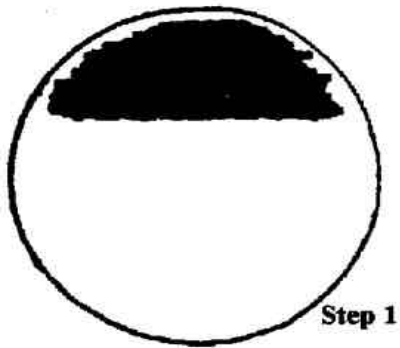
Step 4. Flame the loop and allow it to cool as described above in Step 2.

Step 5. Pass the loop over the streaked portion of the second quadrant two or three times and then streak the material without overlapping over the third quadrant of the plate.

Step 6. Repeat Step 5 to streak the last quadrant.

Most bacteria do not move appreciably from the sites of inoculation but give rise there to clones of bacteria called colonies. Isolated colonies should arise in the third and fourth quadrants depending on the concentration of bacteria in the initial inoculum.

STREAKING BACTERIA FOR COLONY ISOLATION



LABORATORY QUESTIONS:

- 1) What is the purpose of aseptic technique?**
- 2) Why do scientists need to isolate bacterial colonies from a specimen?**
- 3) What is the procedure used to flame a loop?**
- 4) What is the procedure used to streak for bacterial isolation?**
- 5) What are some safety precautions you should take when using a Bunsen burner?**

Answer questions 1-5 in the space below, making sure to number each question!