

Lecture:

Funky Fomites and Aseptic Microbiology Techniques for Bacterial Isolation

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.

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.

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.

Class Notes:

Microbiology Aseptic Techniques:

- Used when isolating bacterial colonies
- Ensures that contaminants are not introduced into the specimen being studied
- Ensures that the person working with a bacterial sample is protected from infectious agents.

Instruments Used When Practicing Aseptic Technique:

- 1) **Flaming/Inoculating Loop:** used for making transfers of bacterial cultures
- 2) **Bunsen Burner:** flammable gas source for sterilization of loop and bacterial source
- 3) **Sparker:** creates the spark needed to ignite the gas from the Bunsen burner
- 4) **Petri dish:** contains media that will act as a nutrient source for bacteria, bacteria are plated onto a petri dish which is then incubated so that bacterial growth may occur
- 5) **Media:** may be liquid or gelatinous: is the nutrient source utilized by bacteria for growth
- 6) **Broth-** liquid nutrient source for bacteria
- 7) **Agar-**gelatinous nutrient source for bacteria
- 8) **Luria Broth:** general type of media that allows all types of bacteria to grow

Selective vs. Differential Media:

- **Selective Media:** has a specific nutrient source or pH that allows for the growth of certain bacteria while inhibiting the growth other bacteria

- **Differential Media:** allows different types of bacteria to grow BUT enables the scientist to distinguish or differentiate between types of bacteria

Bacterial Morphology:

Cocci (circular)

Bacillus (rods)

Spiriochetes (spiral)

Gram Negative vs. Gram Positive Bacteria:

Gram Positive Bacteria: Bacteria that are **gram-positive** are stained dark blue or violet by Gram staining, in contrast to Gram-negative bacteria. The stain is caused by a higher amount of peptidoglycan in the cell wall, which typically lacks the secondary membrane and lipopolysaccharide layer found in other bacteria.

The largest group of Gram-positive bacteria are the Firmicutes; well-known genera include *Bacillus*, *Listeria*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Clostridium*. Other major groups include the Actinobacteria, Planctomycetes, Deinococci, and Thermotogae.

Gram Negative Bacteria:

Bacteria that are **gram-negative** are *not* stained dark blue or violet by Gram staining, in contrast to gram-positive bacteria.

The difference lies in the cell wall of the two types; gram-positive bacteria have a high amount of peptidoglycan in their cell wall which the stain interacts with, while gram-negative bacteria have a cell wall made primarily of lipopolysaccharide. The gram-negative cell wall is similar to a cytoplasmic membrane, typically only a few layers thick and generally much thinner than gram-positive types.

Many species of gram-negative bacteria are pathogenic. This pathogenic capability is usually associated with certain components of their cell walls, particularly the lipopolysaccharide (endotoxin) layer.

The proteobacteria are a major group of gram-negative bacteria, including for instance *Escherichia coli*, *Salmonella*, and other Enterobacteriaceae, *Pseudomonas*, *Moraxella*,

Helicobacter, *Stenotrophomonas*, *Bdellovibrio*, acetic acid bacteria, and a great many others. Other notable groups of gram-negative bacteria include the cyanobacteria, spirochaetes, green sulfur and green non-sulfur bacteria.

Terminology:

Zoonoses- transmission of a disease from an animal to a human

Translucent- a material that one can see through

Turbidity- a material that one cannot see through; usually indicates that bacterial growth has occurred

Flora- natural bacterial inhabitants in the body

Indigenous- native to that habitat, one's normal flora

Pathogenic (noxious)- harmful, disease causing agent

Innocuous- not harmful

Aerobic- requires oxygen to grow

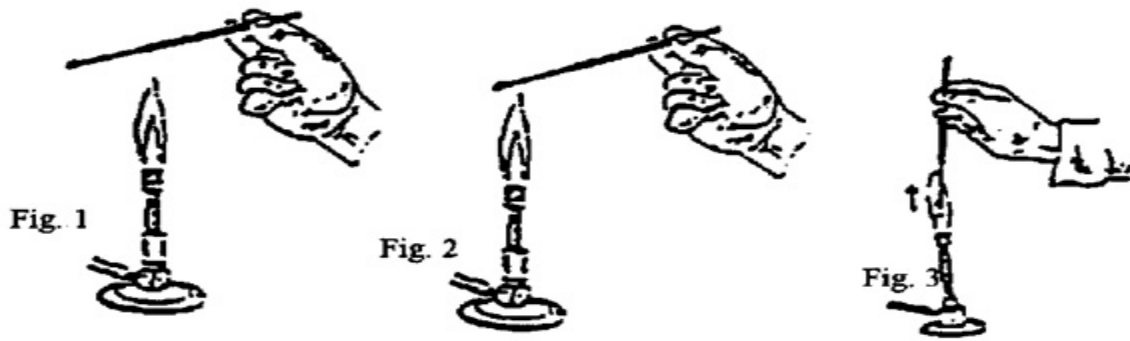
Anaerobic- does not require oxygen to grow

Amorphous- having no definite shape

Laboratory Procedure:

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).



BACTERIAL 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.

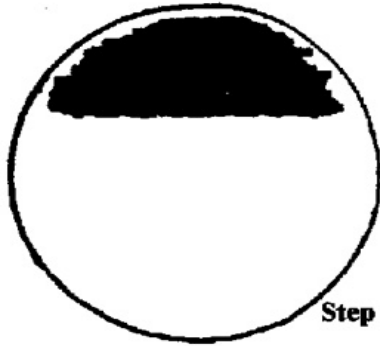
Step 4. Flame the loop and allow it to cool as described above in Step 2.

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



Step 1



Step 3



Step 5



Step 6

List of Possible Fomites for Swabbing:

- toilet bowl rim
- water fountain
- bathroom door handles
- sink handles
- cafeteria lunch counter
- baseball hats
- laboratory bench tops
- desk tops
- gym lockers

LABORATORY REPORT 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?**