



## Funky Fomites and Aseptic Microbiology Techniques for Bacterial Isolation

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### Annotation:

This laboratory exercise includes both a lecture on the purpose of practicing aseptic technique as well as an explanation of how to practice aseptic techniques. Students will actually practice aseptic technique by plating bacteria in order to isolate individual colonies of bacteria. In addition, students will hypothesize which fomites\* they believe will have the most bacterial contamination and take swabs of their fomites to check for bacteria.

*\*Fomites:- any inanimate object such as a desk, table, floor etc. that is capable of being a breeding ground for bacteria*

### Primary Learning Outcome:

Students should understand the significance of aseptic techniques in the clinical and microbiological laboratory. Students should demonstrate knowledge about the tools used in aseptic technique, understand how to isolate bacterial colonies, identify specific types of bacterial organisms, understand differences in bacterial morphologies, differentiate between gram negative and gram positive bacteria, and understand how selective and differential media enable scientists to identify specific bacteria based on the bacteria's characteristics.

### Additional Learning Outcome:

Students should understand how prevalent bacterial contamination is and be able to conceptualize how fomites can be contaminated through physical contact or aerolized contamination. Students should understand how to streak for bacterial isolation and identify individual bacterial colonies.

### Georgia Performance Standards:

SB4

SCSh2

SCSh6

### Materials:

- 1) petri dishes
- 2) agar
- 3) non-virulent bacterial source in broth medium suspension
- 4) wire loops
- 5) Bunsen burner with gas source
- 6) Sterile cotton swabs
- 7) Incubator
- 8) Permanent markers
- 9) Differential and selective media types

**Total Duration:**

1 hr and 30 minutes during first session of experiment

20 minutes during second session of experiment

**Technology Connection:**

Students will view a power point lecture about aseptic techniques and the bacterial streaking for isolation.

**Procedures:**

<b>Step 1</b>	
Description	Aseptic Technique lecture
Duration in hours/minutes	30 minutes
Attachment #1 – Name and description	Funky Fomites and Aseptic Microbiology Techniques for Bacterial Isolation
<b>Step 2</b>	
Description	Demonstration of Laboratory Procedure for Bacterial Streaking and Isolation Using the Quadrant Streaking Method.
Duration in hours/minutes	15 minutes
Attachment #1 – Name and description	Laboratory Procedures Handout
<b>Step 3</b>	
Description	Hands-On Laboratory Bacterial Isolation Activity
Duration in hours/minutes	30 minutes (may vary based on number of available Bunsen burners)
Attachment #1 – Name and description	Laboratory Procedures Handout
<b>Step 4</b>	
Description	Selection of Fomites for Swabbing
Duration in hours/minutes	5 minutes
Attachment #1 – Name and description	List of possible fomites for swabbing
Web Site #1 – URL and annotation	<a href="http://www.epa.gov/nerlcwww/">http://www.epa.gov/nerlcwww/</a>
<b>Step 5</b>	
Description	Swabbing of fomites and plating on agar plates
Duration in hours/minutes	5 minutes
<b>Step 6</b>	
Description	Assignment of Laboratory Report Questions (** teacher must incubate the fomite plates and plates streaked for bacterial isolation and bring them to the following class for the students to see whether or not they isolated bacterial colonies properly and accept or reject their hypotheses about bacterial contamination of various fomites)

Duration in hours/minutes	5 minutes
Attachment #1 – Name and description	Laboratory Write-up Questions
Web Site #1 – URL and annotation	<a href="http://www.microbes.info/">http://www.microbes.info/</a>
Web Site #2 – URL and annotation	<a href="http://www.microbiol.org/">http://www.microbiol.org/</a>

**Assessment:**

- 1) Laboratory Write-up Questions
- 2) Assessment of whether the students were able to isolate individual bacterial colonies after incubation of the petri dishes.
- 3) Assessment of the students' abilities to hypothesize and accept or reject their hypotheses based on analyses of the bacterial growth that occurs from the swabbing of various fomites.

**Extension:**

An unknown bacterial sample could be given and a flow chart could provide students with a pathway to follow in which they could use various differential and selective media to identify their unknown bacteria. This procedure would include the concepts of bacterial isolation as well as the significance of aseptic technique in diagnosing microbial organisms.

**Remediation:**

Bring in properly isolated colonies of bacteria and samples of differential and selective media to show students how aseptic technique and microbiology enables clinicians and scientists to identify organisms.

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

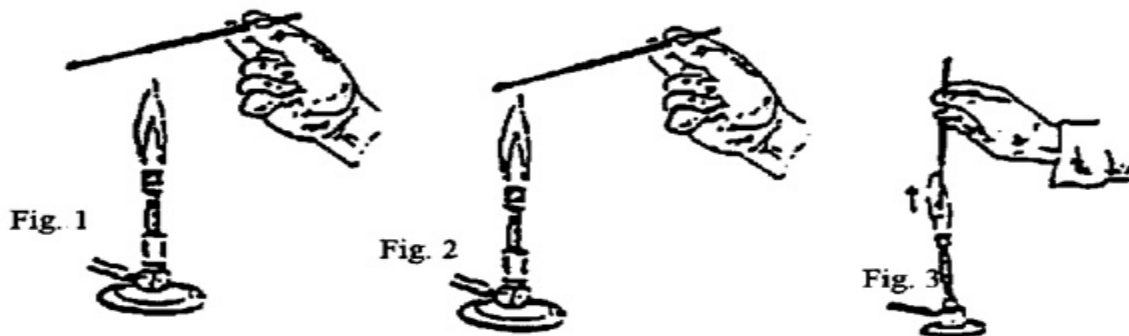
Anaerobic- does not require oxygen to grow

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.

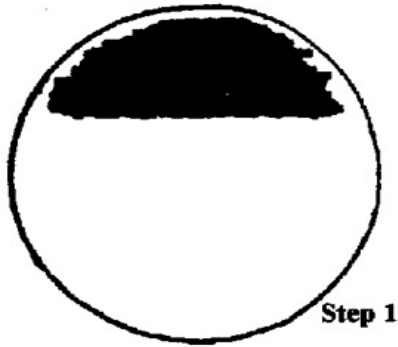
**Step 5.** Pass the loop over the streaked portion of the second quadrant two or three times and then streak the material without overlapping over the third quadrant of the plate.

**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**



**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?**