# Table of Contents

Table of Contents ................................................................. iii  
How to Use This Manual ......................................................... v  
  Purpose of This Manual ...................................................... v  
  Who This Manual is for ...................................................... v  
Equipment Needed for Experiment ...................................... vi  
Organization of This Manual ................................................. vii  
Laboratory Safety ................................................................. viii

Chapter 1: Aseptic Technique ................................................. 3  
  What is Aseptic Technique? ................................................. 3  
  Flaming the Loop ............................................................. 4  
  Keeping Lids Sterile .......................................................... 6

Chapter 2: Starting Off the Experiment .............................. 11  
  Washing your hands ......................................................... 11  
  Cleaning off your lab bench .............................................. 13  
  Organizing your supplies ................................................ 14

Chapter 3: Performing a 4-Quadrant Streak for Isolation of *E. coli* ......................................................... 17  
  Purpose of 4-Quadrant Streak ............................................ 17  
  Part 1: Picking a Colony ................................................... 18  
  Part 2: Streaking the Plate for Isolation ............................. 21  
  Incubation ........................................................................ 25
<table>
<thead>
<tr>
<th>Table of Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index ................................................................. 27</td>
</tr>
<tr>
<td>Citations ............................................................. 29</td>
</tr>
</tbody>
</table>
How to Use This Manual

Purpose of This Manual
This lab manual explains what aseptic technique is, how to prepare a lab station, and how to perform a four-quadrant streak of the bacterium *Escherichia coli* (*E. coli*) while avoiding contamination. Students will become experts on what a four-quadrant streak is, when to perform one, and how to avoid contamination while using this technique.

Who This Manual is for
This manual is for students at the University of North Texas who have completed BIOL 1730 & 1740 or BIOL 1755 and are currently enrolled in BIOL 2041 & 2042. These students are familiar with using a Bunsen burner and know the characteristics of bacteria.
### Equipment Needed for Experiment

The following laboratory equipment is necessary to perform a four-quadrant streak of bacteria for the purpose of isolation.

<table>
<thead>
<tr>
<th>Figure</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wire inoculating loop</td>
</tr>
<tr>
<td>2</td>
<td>Bunsen burner</td>
</tr>
<tr>
<td>3</td>
<td>Striker for Bunsen burner</td>
</tr>
</tbody>
</table>

**Figure 1**
- Wire inoculating loop

**Figure 2**
- Bunsen burner

**Figure 3**
- Striker for Bunsen burner
| Figure 4 | Plate of *E. coli* |
| Figure 5 | TSA plate |

**Organization of This Manual**

This manual includes the following:

- Chapter 1: “Aseptic Technique”
- Chapter 2: “Starting Off the Experiment”
- Chapter 3: “Performing a 4-Quadrant Streak for Isolation of *E. coli*”
- Index
- Citations
Laboratory Safety

Follow these guidelines to create a safe learning environment for you and your fellow students. Disregarding these guidelines will endanger your health and the health of others in the laboratory.

- Wear goggles or safety glasses
- Tie back long hair
- Wear closed toe shoes
- Eating, drinking, or touching contact lenses in lab is prohibited
- Wash hands before and after completing your laboratory work
- Wipe down lab bench with disinfectant before and after each lab to minimize bacterial contamination
- Place agar plates and other contaminated material in **Biohazard trash containers**
  - The biohazard trashcans will be labeled with this symbol
- **Aseptic Bunsen burners are a fire hazard**
  - When there is a potential fire hazard this symbol will be present
Chapter 1: Aseptic Technique
Chapter 1: Aseptic Technique

What is Aseptic Technique?
Aseptic technique is a combination of laboratory methods that keep the worker and work area free of contamination. It also insure culture media contain only the bacterium you are studying. Strategies that keep the experiment aseptic include flaming the inoculating loop and keeping the lid sterile.

Figure 6: Student demonstrating aseptic technique
Flaming the Loop

Flaming the loop sterilizes it, ensuring that you are inoculating only the bacterium of interest.

1. Hold loop in flame of Bunsen burner at a 65° angle

![Figure 7: Flaming the loop](image7.png)

2. Heat until red-hot for 3-5 seconds

![Figure 8: Red-hot loop](image8.png)
Microbiology Lab Manual

3. Cool loop in sterile field around flame for a few seconds

4. Stick loop in agar before picking a colony from the plate
   - The loop usually makes a sizzling sound

Figure 9: Sterile field

Figure 10: Cooling loop in agar
Keeping Lids Sterile

When performing a four-quadrant streak, follow these rules to keep from contaminating the plate:

- Place TSA plate in sterile field around flame of the Bunsen burner

![Figure 11: Convection current](image)

- Take lid off by grabbing the sides and lifting directly upward

![Figure 12: Lifting up lid](image)
Microbiology Lab Manual

- Keep the lid close to plate and in the sterile field at all times

- Do not flip the lid upside down at any time
  - This allows particles from above the flame to get into the lid and contaminate the experiment.

Figure 13: Plate contamination
Chapter 2: Starting Off the Experiment
Chapter 2: Starting Off the Experiment

Washing your hands

1. Wet hands with warm water and apply antibacterial soap

![Antibacterial soap](Figure 14: Antibacterial soap)

2. Scrub hands for 20-30 seconds

![Scrubbing hands](Figure 15: Scrubbing hands)
3. Rinse hands well with water

Figure 16: Rinsing hands

4. Dry hands with unused paper towel

Figure 17: Drying hands with paper towel
Microbiology Lab Manual

Cleaning off your lab bench

1. Wipe entire lab table with Lysol™ disinfectant wipes

![Figure 18: Lysol™ wipes](image)

2. Wait 30 seconds to a minute for table to dry
Organizing your supplies

1. Place Bunsen burner directly in front of you
   - Make sure it is connected to the gas line

2. Place striker beside Bunsen burner

3. Place inoculating loop within arm’s reach

4. Move *E. coli* plate and TSA plate to sterile field beneath flame
Chapter 3: Performing a 4-Quadrant Streak for Isolation of \textit{E. coli}
Chapter 3: Performing a 4-Quadrant Streak for Isolation of *E. coli*

**Purpose of 4-Quadrant Streak**
The purpose of performing a 4-quadrant streak is to isolate pure colonies of bacteria.

*Figure 20: Isolated colonies on plate*
Part 1: Picking a Colony

1. Divide TSA plate into four quadrants

![Figure 21: Labeled TSA plate](image)

2. Sterilize and cool loop

![Figure 22: Sterilizing loop](image)
3. Remove lid from *E. coli*
   - Hint: Using non-dominant hand to remove lid is most effective

4. Cool loop in agar

Figure 23: Removing lid

Figure 24: Cooling loop
Chapter 3

5. Pick isolated colony on plate with loop
   o Hint: More isolated colonies tend to be more pure

Figure 25: Isolated colonies

6. Close lid and replace with TSA plate
Part 2: Streaking the Plate for Isolation

1. Place TSA plate on rack

2. Remove lid with thumb and middle finger
3. Streak quadrant 1 on TSA plate

4. Put lid of plate back on

5. Sterilize and cool loop
6. Streak quadrant 2 on TSA plate

Figure 29: Quadrant 2

7. Repeat steps 3 & 4

8. Streak quadrant 3 on TSA plate

Figure 30: Quadrant 3
9. Repeat steps 3 & 4

10. Streak quadrant 4 on TSA plate

![Figure 31: Quadrant 4](image)

11. Repeat step 4

12. Incubate plate at 37°C

![Figure 32: Incubation](image)
Incubation

Incubation is a very important part of developing pure, isolated colonies. Store the *E. coli* plate in an environment that is 37°C and check after 24 hours. If the bacterium does not develop colonies in the agar by this time, wait another 24 hours and check on the plate.
Index

Aseptic, 3, 4, 5, 3
Bunsen burner, 1, 2, 4, 6, 14
Colony, 20
E. coli, 1, 3, 14, 15, 17, 19, 25
Four-quadrant streak, 1, 2, 6

Loop, 2, 3, 4, 5, 14, 18, 19, 20, 22
Plate, 3, 5, 6, 7, 14, 18, 20, 21, 22, 23, 24, 25
TSA, 3, 6, 14, 18, 20, 21, 23, 24
Citations
