

Pipetting & Aseptic Technique

Grade Level: 11 & 12
Summer
Intern

Subject: Biotechnology / Molecular
Biology/ Techniques

Prepared By: Larry Cosenza
C2 Biotechnologies, LLC
lcosenza@c2biotechnologies.com

<p>Overview & Purpose</p> <p>Pipetting small volume skills and basic aseptic technique. These skills are basic requirements for all life science laboratories. Aseptic technique prevents cross contamination under BLS1 and infection or death under BLS4 levels of containment.</p>	<p>Education Standards Addressed</p>
--	---

	Teacher Guide	Student Guide	
<p>Objectives (Specify skills/information that will be learned.)</p>	<p>Understand bio-safety levels and responses to small accidents. Physical dexterity for pipetting small volumes of 1 μl to 1000 μl (1 ml).</p>	<p>To pipette small volumes of chemicals and biologics safely and efficiently.</p>	<p>Materials Needed</p> <ul style="list-style-type: none"> • Pipettes • Eppendorf tubes • Hood • SOP
<p>Information (Give and/or demonstrate necessary information)</p>	<p>Definitions of Bio-Safety Levels (BSL) 1 -4. Demonstrate how to operate pipettes – setting volume-taking sample – dispensing sample.</p>	<p>Perform serial dilutions. Perform serial dilutions with mock hazardous biologic or radioactive sample.</p>	
<p>Verification (Steps to check for student understanding)</p>	<p>Play game “Survival”. Use mock hazardous samples for testing.</p>	<p>All play. Define BSL for specific mock sample. Prepare serial dilution with mock sample.</p>	<p>Other Resources (e.g. Web, books, etc.) http://www.cdc.gov/biosafety/</p>
<p>Activity (Describe the independent activity to reinforce this lesson)</p>	<p>Exercises to understand difference between small and large volumes. Define BSL1-4. Give examples of biologics.</p>	<p>Laboratory note books. Generate a table of conversion formula for volume. List BSL s 1-4 and correlate given biologics with proper BSL.</p>	
<p>Summary</p>	<p>Understanding individual actions affect group.</p>	<p>Best technique and common sense is safest approach.</p>	<p>Additional Notes</p>

Playing the “Survival” game.

Objective: to survive routine examination and serial dilution of a biologic / chemical specimen.

Rules: Everyone plays. An individual is handed a **mock** labeled eppendorf tube and asked to perform a series of simple manipulations.

1. Streak a plate
2. A 1:10 serial dilution
3. Gram stain

Mock Specimens:

1. 10 ul of Militarized anthrax in eppendorf tube.
2. 10 ul of I¹²⁹ labeled specimen.
3. 10 ul of total RNA extract from polio virus specimen.

Instructor:

What to look for. Each of these mock specimens is used to exemplify proper or improper technique and test individuals knowledge of aseptic techniques. These samples also are used to demonstrate that everyone in the laboratory is responsible and affected by the actions of any other individual in the laboratory.

Sample 1. Testing aseptic technique and laboratory awareness. Airborne infection, mock sample has 90% lethality rate. Instructor is looking potential release of organism from open tube outside of hood. End result - All individuals in room are infected, laboratory is quarantined. No winners.

Discussion: Bio-safety levels, proper aseptic technique, how individual actions can affect all laboratory members.

Sample 2. Testing aseptic technique and laboratory awareness. I¹²⁹ is a volatile radioactive gamma emitter. Easily inhaled and the resultant material concentrates in thyroid. Generally a labeled sample is not volatile if all free I¹²⁹ has been removed. Instructor is looking for sloppy technique and spills. If tube is opened outside of hood individual is contaminated by inhaling free volatile material. Small spills or splattering of material on clothing leads to contamination. Improper handling can lead to radiation contamination through laboratory which can be translated to other individuals. Secondary contamination can leave laboratory and contaminate friends and family. Example, radioactive contaminated pants are washed in house hold laundry. End result – All individuals in room are contaminated laboratory is heavily fined by OSHA and shut down. No winners.

Discussion: Bio-safety levels, proper aseptic technique, how individual actions can affect all laboratory members inside and outside of the laboratory.

Sample 3. Testing aseptic technique and laboratory awareness. RNA from an RNA virus is as infectious as the whole virus. Instructor is looking for sloppy technique where small spill or splattering infects individual. The individual becomes a vector for the spread of infection. Infected individual is ground zero, transmits infection to rest of laboratory, family and friends. End result- All individuals in room are contaminated. No winners.

Discussion: Bio-safety levels, proper aseptic technique, laboratory protective gear, how individual actions can affect all laboratory members inside and outside of the laboratory.

Summary

It is important to be aware of all projects and procedures in a laboratory. The best defense knowing and understanding where you are, what you are doing, who else is in the laboratory and what you are working with.