

Exercise 1.1 – Safety in the Microbiology Laboratory

LEARNING OUTCOMES

1. Identify best practices and safety regulations for the microbiology laboratory.
 2. List and compare the four biological safety levels.
 3. Describe proper disposal of biological and non-biological laboratory waste.
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You probably have taken a biology or chemistry course and are already familiar with good laboratory practices and safety protocols. The microbiology lab is a bit different because there are strict guidelines for handling and disposing of live organisms.

In the United States, multiple organizations on the local, state, and federal level work together to monitor and ensure laboratory safety. The Centers for Disease Control and Prevention (CDC) provide guidance to clinical, research, public health, and educational laboratories that handle potentially infectious agents, such as viruses and bacteria, that can spread among hosts.

Biological Safety Levels

The CDC has established four biological safety levels (BSL) to classify microorganisms based on each agent's infectivity, ease of transmission, and potential disease severity, as well as the type of work being done with the agent (Figure 1.2). Each BSL requires a different level of biocontainment to prevent contamination and spread of infectious agents to laboratory personnel and, ultimately, the community.

BSL-1 requires the fewest precautions because it applies to situations with the lowest risk for microbial infection in healthy adults. These include nonpathogenic strains of *Escherichia coli* and environmental bacteria such as *Bacillus subtilis*. Laboratory workers may work with these agents at an open laboratory bench wearing personal protective equipment (PPE) such as a laboratory coat, goggles, and gloves, as needed.

Agents classified as BSL-2 include those that include organisms that may be potentially pathogenic or infectious, and particularly those that may aerosolize or disperse through the air. Working with BSL-2 bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes*, or viruses like hepatitis, mumps, and measles, require additional precautions beyond those of BSL-1, including restricted access to the laboratory, required PPE, and the use of biological safety cabinets for certain organisms.

BSL-3 agents have the potential to cause lethal infections by inhalation. These may include pathogens such as *Mycobacterium tuberculosis*, *Bacillus anthracis*, human immunodeficiency virus (HIV), and coronavirus (SARS-CoV-2). Because of the serious nature of the infections caused by BSL-3 agents, laboratories working with them require restricted access and are equipped with directional airflow which cannot be recirculated. Laboratory personnel always wear a respirator and handle microbes in a biological safety cabinet.

BSL-4 agents are the most dangerous and often fatal. These microbes are easily transmitted by inhalation and cause infections for which there are no treatments or vaccinations. Examples include Ebola and smallpox viruses. There are only a small number of laboratories in the United States and around the world appropriately equipped to work with these agents.

Biosafety Levels			
Biological Safety Levels	Description	Examples	CDC Classification
BSL-4	Microbes are dangerous and exotic, posing a high risk of aerosol-transmitted infections, which are frequently fatal without treatment or vaccines. Few labs are at this level.	Ebola and Marburg viruses	BSL-4 high-risk microbes
BSL-3	Microbes are indigenous or exotic and cause serious or potentially lethal diseases through respiratory transmission.	<i>Mycobacterium tuberculosis</i>	BSL-3
BSL-2	Microbes are typically indigenous and are associated with diseases of varying severity. They pose moderate risk to workers and the environment.	<i>Staphylococcus aureus</i>	BSL-2
BSL-1	Microbes are not known to cause disease in healthy hosts and pose minimal risk to workers and the environment.	Nonpathogenic strains of <i>Escherichia coli</i>	BSL-1 low-risk microbes

Figure 1.2: The CDC classifies infectious agents into four biosafety levels based on potential risk to laboratory personnel and the community. Each level requires a progressively greater level of precaution.

Best Practices in the Microbiology Laboratory

Departmental safety regulations will be reviewed during the first lab period. Your instructor will provide specific instructions each week for working with microorganisms, but some general points below are relevant for all microbiology sections. Please read them carefully.

1. Live bacteria, fungi, and protists in concentrated numbers are used for most exercises. Although these microorganisms are typically found in the environment or our bodies, all should be treated as potential pathogens and handled with care.
2. Wear suitable clothing and shoes for working in a laboratory and always use appropriate personal protective equipment when working with cultures.
3. Wash your hands and change gloves frequently and keep pens/pencils and fingers away from your mouth and face.
4. Disinfect the top of your work area before and after lab, wiping the back of your chair and any personal items used during lab such as your notebook, laptop, etc., as well.
5. Never pour anything in the sink or place in the regular lab trash without approval. Microbial cultures and other biological waste must be sterilized by autoclaving prior to disposal. Your instructor will provide specific instructions for clean-up each week.
6. Food containers, beverage bottles, gum, lip balm, and cell phones are all things that are used in or near your mouth. These can become contaminated with microbes and therefore are not permitted in the lab at any time.
7. For most exercises, an item that generates intense heat or an open flame is used. Never let go of a metal inoculating loop while using the incinerator and never leave an open flame unattended. Keep electrical cords from contact with hot plates and incinerators.
8. Notify your instructor immediately of any safety incident no matter how minor. Never attempt to clean up a culture spill or broken glass on your own; there are special protocols for these types of accidents that must be followed.
9. The caps of test tubes that contain bacteria are often loose, so be careful when picking up tubes and always use a test tube rack.
10. Most materials are sterile and must be kept free from contamination, so keep in mind that when an item is opened it is no longer sterile. Do not return any used or contaminated items to a rack or container with sterile materials. Likewise, do not place used pipettes or swabs back in their wrapper or in the lab trash bin.
11. Finally, always leave your lab bench clean and neat for the student who follows you.

Microbiology Laboratory

Best Practices

Always wear appropriate personal protective equipment

Disinfect bench before and after lab

Have proper attire and tie hair back

Wash hands frequently

Keep cell phones away

Only necessary materials on bench top

No food, drinks, or gum in lab at any time

Follow safe disposal guidelines for all materials, cultures, and chemicals

Notify instructor of any accident immediately, no matter how minor

