

# BIOSAFETY LEVELS

Biological research or production labs may work with potentially harmful biological agents which can cause disease in people. These can include pathogens, such as viruses, bacteria, fungi or parasites, as well as some toxic substances. Labs designed to work with hazardous biological agents must have containment features and safe work practices in place to protect laboratory personnel who work with these agents, and to prevent these agents from escaping the lab and harming the community or the environment. These precautions and practices are organized into **biosafety levels** based on the risks associated with the biological agents being used and the work being done in the lab.

In the United States, biosafety levels are defined by the Centers for Disease Control (CDC) and the National Institutes of Health (NIH). They outline four biosafety levels, or BSLs, that are ranked from 1 to 4 based on the level of risk. BSL-1 precautions are used for the least hazardous biological agents and practices, while BSL-4 precautions are for the most hazardous. BSLs are chosen based on considerations such as what the biological agent is, how much of it is being used, how severe infection could be, how easily the agent could be transmitted, the availability of prevention or treatment for exposure to the agent, the level of exposure risk created by laboratory processes, and worker training and skill levels.

## Biosafety Level-1

Biosafety level one is the lowest level of precautions. BSL-1 practices are used for work with agents that pose a minimal risk to workers or the environment and do not typically cause disease in healthy adults. Common examples of agents used in BSL-1 laboratory environments are non-pathogenic strains of *E. coli* and *Bacillus subtilis*. While these organisms do present some small amount of risk, standard microbiological practices are usually enough to protect people from them.

Standard microbiological practices include:

- Hand washing
- Standard personal protective equipment (gloves, lab coat or gown, and eye protection)
- Biohazard signs
- Controlled access to the facility when infectious agents are present
- Shielding from splashes or aerosols
- Decontaminating surfaces and equipment at least daily
- Immediate cleanup and decontamination of spills
- Safe handling of sharps
- Prohibiting mouth pipetting
- Prohibiting food, drink and smoking materials in the lab
- Decontamination of infectious materials prior to disposal

BSL-1 facilities do not need special containment equipment. Work typically takes place on open bench tops, and the facility does not need to be isolated from surrounding facilities. Because these labs are relatively safe and easy to maintain, they can be used as teaching spaces for workers and students with low levels of training, such as high school biology classes.

## **Biosafety Level-2**

Biosafety level two is for moderate biological hazards. BSL-2 practices can be used for work involving agents that are associated with human diseases (pathogenic or infectious organisms) that pose a moderate hazard to personnel and the environment, such as HIV and the bacteria that cause staph infections. Work that involves human blood or cell lines is also considered a minimum of BSL-2. In addition to standard microbiological practices, BSL-2 laboratories must follow these additional practices:

- Lab personnel are trained to handle pathogenic agents and are supervised by scientists with advanced training
- Biosafety cabinets or other physical containment for all procedures that can generate infectious aerosols or splashes
- Autoclave or other method of decontamination for proper disposal
- Eyewash station
- Lockable doors and more controlled facility access
- Extra care is taken to control routes of exposure, including advanced techniques for handling contaminated sharps
- Immunizations are provided to lab personnel when appropriate
- Additional PPE, such as face shields, may be necessary
- A lab-specific biosafety manual that outlines the necessary controls and practices for the work performed in that lab

BSL-2 facilities will need moderate containment methods such as Class II biosafety cabinets. All surfaces in the lab must be easy to clean and decontaminate effectively. Carpets and rugs are not allowed, and windows must be fitted with screens.

## **Biosafety Level-3**

Biosafety level three is for serious biological hazards. BSL-3 practices are appropriate for work involving agents that can cause serious or potentially fatal disease through inhalation. Examples of agents commonly used in BSL-3 work include yellow fever virus, SARS coronavirus, and tuberculosis bacteria. BSL-3 work is often strictly controlled by government agencies, and labs may need to be registered.

BSL-3 labs must follow all of the same practices as BSL-1 and BSL-2 labs. In addition, BSL-3 labs must incorporate stricter measures including:

- Baseline medical testing and ongoing medical surveillance for all workers with potential exposure to infectious agents
- Full body PPE such as wraparound gowns, scrub suits or coveralls
- Respiratory protection may be required
- All work with infectious agents must be performed in an appropriate BSC or other physical containment device
- Access is restricted and controlled at all times
- More stringent control of contaminated waste, equipment and lab clothing
- A set of two separate, self-closing doors, separated from general building corridors

BSL-3 facilities must have more advanced containment methods, including specialized ventilation that directs air from clean areas towards areas where

infectious agents are present, and does not allow air to recirculate unless it runs through a HEPA filter first. Windows must be sealed so that airborne particles cannot escape.

### **Biosafety Level-4**

Biosafety level four is the highest level of precautions. BSL-4 practices are used for work with agents that are very easily transmitted and cause serious or fatal diseases for which there are no vaccines or treatments, such as the Ebola virus and the virus that causes smallpox. These facilities are rare and are highly regulated.

In addition to the precautions used in BSL-1, 2 and 3 facilities, BSL-4 labs must use practices such as:

- A complete clothing change before entering the lab, and a decontamination shower before exiting
- Decontamination of all materials before leaving the facility
- Strictly-controlled access and records of all persons entering and exiting the facility
- An airlock entrance
- Perform all work in a class III biosafety cabinet, or a combination of a class I or II biosafety cabinet and a positive-pressure full-body suit with an air-supplied respirator

BSL-4 work must typically take place in a dedicated building or a completely isolated area of the facility with dedicated air intake and exhaust, and dedicated vacuum lines and decontamination systems. Air exhaust and used water must pass through HEPA filtration before leaving the facility.

More information about biosafety levels and containment practices can be found in the CDC publication, Biosafety in Microbiological and Biomedical Laboratories, 5th Edition.



Biological Safety Levels (BSL) are a series of protections relegated to autoclave-related activities that take place in particular biological labs. They are individual safeguards designed to protect laboratory personnel, as well as the surrounding environment and community.

These levels, which are ranked from one to four, are selected based on the agents or organisms that are being researched or worked on in any given laboratory setting. For example, a basic lab setting specializing in the research of nonlethal agents that pose a minimal potential threat to lab workers and the environment are generally considered BSL-1—the lowest biosafety lab level. A specialized research laboratory that deals with potentially deadly infectious agents like Ebola would be designated as BSL-4—the highest and most stringent level.

The Centers for Disease Control and Prevention(CDC) sets BSL lab levels as a way of exhibiting specific controls for the containment of microbes and biological agents. Each BSL lab level builds upon on the previous level—thereby creating layer upon layer of constraints and barriers. These lab levels are determined by the following

- Risks related to containment
- Severity of infection
- Transmissibility
- Nature of the work conducted
- Origin of the microbe
- Agent in question
- Route of exposure

The reason biosafety levels are so important is because they dictate the type of work practices that are allowed to take place in a lab setting. They also heavily influence the overall design of the facility in question, as well as the type of specialized safety equipment used within it.

The following is an explanation of each biosafety level—what they mean and how they differ in safety measures and best practices.

### **[Download our BSL Quick Reference Guide Here >](#)**

## **BSL-1**

As the lowest of the four, biosafety level 1 applies to laboratory settings in which personnel work with low-risk microbes that pose little to no threat of infection in healthy adults. An example of a microbe that is typically worked with at a BSL-1 is a nonpathogenic strain of *E. coli*.

This laboratory setting typically consists of research taking place on benches without the use of special contaminant equipment. A BSL-1 lab, which is not required to be isolated from surrounding facilities, houses activities that require only standard microbial practices, such as:

- Mechanical pipetting only (no mouth pipetting allowed)
- Safe sharps handling
- Avoidance of splashes or aerosols
- Daily decontamination of all work surfaces when work is complete
- Hand washing
- Prohibition of food, drink and smoking materials in lab setting
- Personal protective equipment, such as; eye protection, gloves and a lab coat or gown
- Biohazard signs

BSL-1 labs also requires immediate decontamination after spills. Infection materials are also decontaminated prior to disposal, generally through the use of an autoclave.

## BSL-2

This biosafety level covers laboratories that work with agents associated with human diseases (i.e. pathogenic or infectious organisms) that pose a moderate health hazard. Examples of agents typically worked with in a BSL-2 include equine encephalitis viruses and HIV, as well as *Staphylococcus aureus* (staph infections).

BSL-2 laboratories maintain the same standard microbial practices as BSL-1 labs, but also includes enhanced measures due to the potential risk of the aforementioned microbes. Personnel working in BSL-2 labs are expected to take even greater care to prevent injuries such as cuts and other breaches of the skin, as well as ingestion and mucous membrane exposures.

In addition to BSL 1 expectation, the following practices are required in a BSL 2 lab setting:

- Appropriate personal protective equipment (PPE) must be worn, including lab coats and gloves. Eye protection and face shields can also be worn, as needed.
- All procedures that can cause infection from aerosols or splashes are performed within a biological safety cabinet (BSC).
- An autoclave or an alternative method of decontamination is available for proper disposals.
- The laboratory has self-closing, lockable doors.
- A sink and eyewash station should be readily available.
- Biohazard warning signs

Access to a BSL-2 lab is far more restrictive than a BSL-1 lab. Outside personnel, or those with an increased risk of contamination, are often restricted from entering when work is being conducted.

## BSL-3

Again building upon the two prior biosafety levels, a BSL-3 laboratory typically includes work on microbes that are either indigenous or exotic, and can cause serious or potentially lethal disease through inhalation. Examples of microbes worked with in a BSL-3 includes; yellow fever, West Nile virus, and the bacteria that causes tuberculosis.

The microbes are so serious that the work is often strictly controlled and registered with the appropriate government agencies. Laboratory personnel are also under medical surveillance and could receive immunizations for microbes they work with.

Common requirements in a BSL-3 laboratory include:

- Standard personal protective equipment must be worn, and respirators might be required
- Solid-front wraparound gowns, scrub suits or coveralls are often required
- All work with microbes must be performed within an appropriate BSC
- Access hands-free sink and eyewash are available near the exit
- Sustained directional airflow to draw air into the laboratory from clean areas towards potentially contaminated areas (Exhaust air cannot be re-circulated)
- A self closing set of locking doors with access away from general building corridors

Access to a BSL-3 laboratory is restricted and controlled at all times.

## BSL-4

BSL-4 labs are rare. However some do exist in a small number of places in the US and around the world. As the highest level of biological safety, a BSL-4 lab consists of work with highly dangerous and exotic microbes. Infections caused by these types of microbes are frequently

fatal, and come without treatment or vaccines. Two examples of such microbes include Ebola and Marburg viruses.

In addition to BSL-3 considerations, BSL-4 laboratories have the following containment requirements:

- Personnel are required to change clothing before entering, shower upon exiting
- Decontamination of all materials before exiting
- Personnel must wear appropriate personal protective equipment from prior BSL levels, as well as a full body, air-supplied, positive pressuresuit
- A Class III biological safety cabinet

A BSL-4 laboratory is extremely isolated—often located in a separate building or in an isolated and restricted zone of the building. The laboratory also features a dedicated supply and exhaust air, as well as vacuum lines and decontamination systems.

Knowing the difference in biosafety lab levels and their corresponding safety requirements is imperative for anyone working with microbes in a lab setting.

**Get Your Safety Tips Checklist for BSL Labs 1-4 Here >**

Biosafety Level	BSL-1	BSL-2	BSL-3	BSL-4
Description	<ul style="list-style-type: none"> <li>• No Containment</li> <li>• Defined organisms</li> <li>• Unlikely to cause disease</li> </ul>	<ul style="list-style-type: none"> <li>• Containment</li> <li>• Moderate Risk</li> <li>• Disease of varying severity</li> </ul>	<ul style="list-style-type: none"> <li>• High Containment</li> <li>• Aerosol Transmission</li> <li>• Serious/Potentially lethal disease</li> </ul>	<ul style="list-style-type: none"> <li>• Max Containment</li> <li>• "Exotic," High-Risk Agents</li> <li>• Life-threatening disease</li> </ul>
Sample Organisms	E.Coli	Influenza, HIV, Lyme Disease	Tuberculosis	Ebola Virus
Pathogen Type	Agents that present minimal potential hazard to personnel & the environment.	Agents associated with human disease & pose moderate hazards to personnel & the environment.	Indigenous or exotic agents, agents that present a potential for aerosol transmission, & agents causing serious or potentially lethal disease.	Dangerous & exotic agents that pose a high risk of aerosol-transmitted laboratory infections & life-threatening disease.
Autoclave Requirements	None	None	Pass-thru autoclave with Bioseal required in laboratory room.	Pass-thru autoclave with Bioseal required in laboratory room.