

## Biosafety Levels 1, 2, 3 & 4

**Biological Safety Levels (BSL)** are a series of protections relegated to the 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.

### 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.

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## 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.

List of common BSL-2 level agents

Searchable Database

- [Risk Group Classification for Infectious Agents](#) (American Biological Safety Association)
- [Material Safety Data Sheets for Biological Materials](#) (Health Canada)

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## 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.

List of Biological Safety Level III agents.

Searchable Database

- [Risk Group Classification for Infectious Agents](#) (American Biological Safety Association)

- [Material Safety Data Sheets for Biological Materials](#) (Health Canada)

## 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 pressure](#) suit
- 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.

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 and the environment.	Agents associated with human disease and pose moderate hazards to personnel and the environment.	Indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease.	Dangerous and exotic agents that pose a high risk of aerosol-transmitted laboratory infections and 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.

Figure 1. Summary of Biological Agents.