

3.4. Biosafety Containment Levels

Four levels of biosafety are defined in the publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, published by the CDC and NIH. The levels, designated in ascending order by degree of protection provided to personnel, the environment, and the community, are combinations of laboratory practices, safety equipment, and laboratory facilities (see [Appendices E1-3](#)). Most microbiological work at Indiana University is conducted at BSL-1 or BSL-2 containment. The Indiana University Biosafety Manual supersedes the information in the BMBL [Appendices E1-3](#) and must be followed should information differ.

Below is a summary of each biosafety level. Detailed criteria for each level are described in [Appendix E1-3](#).

3.4.1. [Biosafety Level 1](#)

Suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science. Personal protective equipment shall be used as appropriate, including lab coats and gloves. Eye protection shall be used when splashing is likely.

Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

3.4.2. [Biosafety Level 2](#)

Practices, equipment, and facility design and construction are applicable to research, clinical, diagnostic, and teaching laboratories in which work is done with moderate-risk agents that are present in the community. Hepatitis B virus, HIV, salmonellae typhi, and *Toxoplasma gondii*. are representative of microorganisms assigned to this containment level. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines (Laboratory personnel working with human-derived materials shall refer to the [OSHA Bloodborne Pathogen Standard](#) for specific required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution shall be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment such as a biological safety cabinet (BSC) or safety centrifuge cups. Personal protective equipment shall be used as appropriate, including lab coats and gloves. Eye protection shall be used when splashing is likely.

Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

3.4.3. Biosafety Level 2+ BSL3 Practices

Used to describe biocontainment within a Biosafety Level 2 laboratory but using specific Biosafety Level 3 practices. This is not intended to be used as a substitute for Biosafety Level 3 with any Risk Group 3 biohazards. The final determination of this biocontainment is based on a risk assessment of the research planned. The risk assessment and review by the IBC may determine that safety practices above BSL-2 are required, but the research does not warrant the more complex BSL-3 laboratory suite.

No inclusive list of BSL-2+ viral vectors, microorganisms, biohazards, or experimental designs exists and decisions on IU research biocontainment is based on case by case risk assessment. The main focus of BSL-2 + BSL-3 Practices is a reduction in exposure to aerosols and/or particularly hazardous agents that do not quite meet the definition of Risk Group 3 biohazards. Some examples of experiments that may fall under the above definition would be:

- Viral vectors that have inserts of oncogenes or other gene products that may be toxic, particularly if injections are involved;
- Specific multi-drug resistant BSL-2 bacteria;
- High concentrations of Risk Group 2 viruses represented as inhalation hazards; and
- Large volumes of viral vectors, i.e., greater than 10 liters.

3.4.4. Biosafety Level 3

Practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations are performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

3.4.5. Biosafety Level 4

Practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. There are no BSL-4 level laboratories at Indiana University.

Summary of Recommended Biosafety Levels

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	<ul style="list-style-type: none"> • PPE: Laboratory coats; latex or nitrile disposable gloves; eye/face protection as needed 	Laboratory bench and sink required. Autoclave available
2	<ul style="list-style-type: none"> • Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, and mucous membrane exposure 	BSL-1 practices plus: <ul style="list-style-type: none"> • Limited access • Biohazard warning signs • “Sharps” precautions • Biosafety Manual defining any needed waste decontamination or medical evaluation program 	BSL-1 Primary barriers plus: <ul style="list-style-type: none"> • Class I or II BSCs or other physical containment devices or appropriate PPE used for all manipulations of agents that cause splashes or aerosols of infectious materials 	BSL-1 plus: <ul style="list-style-type: none"> • Recommended negative differential pressure • Readily available eyewash
3	<ul style="list-style-type: none"> • Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences 	BSL-2 practices plus: <ul style="list-style-type: none"> • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum 	Primary barriers: <ul style="list-style-type: none"> • Class I or II BSCs or other physical containment devices used for all open manipulation of agents • PPE: <ul style="list-style-type: none"> • Protective laboratory clothing; latex or nitrile disposable gloves; respiratory protection as needed 	BSL-2 plus: <ul style="list-style-type: none"> • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory
4	<ul style="list-style-type: none"> • Dangerous/exotic agents which pose high risk of life-threatening disease • Aerosol transmitted laboratory infections have occurred; or related agents with unknown risk of transmission 	BSL-3 practices plus: <ul style="list-style-type: none"> • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility 	Primary barriers: <ul style="list-style-type: none"> • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit 	BSL-3 plus: <ul style="list-style-type: none"> • Separate building or isolated zone • Dedicated supply and exhaust, vacuum • Decontamination systems • Other requirements outlined in the BMBL