The term **containment** is used to describe safe methods for managing biohazard agents in the laboratory environment. The three essential elements of containment are (1) laboratory practice and technique, (2) safety equipment, and (3) facility design. The purpose of containment is to reduce exposure of laboratory workers and others to potentially hazard agents and prevent the escape of these agents into the outside environment.

Research or teaching activities involving biohazard agents of RG 2 or higher can only be conducted with prior approval of the IBC. The elements of a safety plan have been discussed previously. The NIH/CDC manual *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* (5th Edition) provides guidance for the appropriate containment of biohazard work. The biosafety levels are based on the probability of occupationally-acquired infections resulting from the handling of specific agents in the laboratory.

CDC describes **four biosafety levels** (BSLs) which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations conducted, the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity. The recommended biosafety level for an organism represents the conditions under which the agent can be ordinarily handled safely. When specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified.

**Biosafety Level 1** is appropriate for work done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. It represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.
Biosafety Level 2 is applicable to work done with a broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. Agents can be used safely on the open bench, provided the potential for producing splashes or aerosols is low. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures or ingestion of infectious materials. Procedures with high aerosol or splash potential must be conducted in primary containment equipment such as biosafety cabinets. Primary barriers such as splash shields, face protection, gowns and gloves should be used as appropriate. Secondary barriers such as hand washing and waste decontamination facilities must be available.

Biosafety Level 3 is applicable to work done with indigenous or exotic agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection. Primary hazards to personnel working with these agents (i.e., Mycobacterium tuberculosis, St. Louis encephalitis virus and Coxiella burnetii) include auto-inoculation, ingestion and exposure to infectious aerosols. Greater emphasis is placed on primary and secondary barriers to protect personnel in adjoining areas, the community and the environment from exposure to infectious aerosols. For example, all laboratory manipulations should be performed in biological safety cabinets or other enclosed equipment. Secondary barriers include controlled access to the laboratory and a specialized ventilation system to prevent the release of infectious agents in the event an accidental release occurs in the laboratory.

Biosafety Level 4 is 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. Agents with close or identical antigenic relationship to Biosafety Level 4 agents should also be handled at this level. Primary hazards to workers include respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets and auto-inoculation. The facility is generally a separate building or a completely isolated zone within a complex with
specialized ventilation and waste management systems to prevent the release of viable agents to the environment. All manipulations of potentially infected materials and isolates pose a high risk of exposure and infection to personnel, the community and the environment. Isolation of aerosolized infectious materials is accomplished primarily by working in a Class III biological safety cabinet or a full-body, air-supplied positive pressure personnel suit.

**Vertebrate Animal Biosafety Levels**

There are four animal biosafety levels (ABSLs), designated Animal Biosafety Level 1 through 4, for work with infectious agents in mammals. The levels are combinations of practices, safety equipment and facilities for experiments on animals infected with agents that produce or may produce human infection. In general, the biosafety level recommended for working with an infectious agent in vivo and in vitro is comparable.

**Animal Biosafety Level 1** is suitable for work involving well characterized agents that are not known to cause disease in healthy human adults, and that are of minimal potential hazard to laboratory personnel and the environment.

**Animal Biosafety Level 2** is suitable for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

**Animal Biosafety Level 3** is suitable for work with animals infected with indigenous or exotic agents that present the potential of aerosol transmission and of causing serious or potentially lethal disease.

**Animal Biosafety Level 4** is suitable for addressing dangerous and exotic agents that pose high risk of like threatening disease, aerosol transmission, or related agents with unknown risk of transmission.

Complete descriptions of all [Biosafety Levels](#) and [Animal Biosafety Levels](#) are outlined in the NIH/CDC manual *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* (5th Edition). [The BMBL](#)
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provides minimum guidelines for containment of biohazards. The university containment requirements and laboratory practices may be more stringent. When in doubt, contact the Institutional Biosafety Officer for confirmation of university requirements.

Research at the university is limited to Biosafety Levels 1, 2 or 3. Infectious materials must be clearly identified and stored in such a manner as to preclude accidental exposure. This normally includes double containment and labeling of the storage freezer/refrigerator/liquid nitrogen tank.

VI.1. Laboratory-acquired Infections

A number of infectious agents have been documented as causes of laboratory-acquired infections. Included in the list are bacterial, viral, chlamidial and rickettsial, and parasitic organisms.

VI.2. Laboratory Practices

The most important element of containment is strict adherence to standard microbiological practice and techniques. Persons working with biohazard agents or infected materials shall be aware of potential hazards and shall be trained and proficient in the practices and techniques required for safe handling. When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures such as safety equipment and facility design must be used.

VI.3. Safety Equipment (Primary Barriers)

Safety equipment includes personal protective equipment, biological safety cabinets, sealed, leak proof containers, and other engineering controls designed to prevent or minimize exposures to hazardous biological materials. The use of vaccines, if available, is encouraged or in some instances specified to provide an increased level of personal protection.
VI.3.1. Biological Safety Cabinets (BSC)

The biological safety cabinet is the principal device used to provide containment of infectious splashes or aerosols. Biological Safety Cabinets are designed to protect the worker, the integrity of the experiment, and the environment. There are three types of biological safety cabinets: Class I, Class II and Class III.

CDC and NIH have published a document entitled Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets that is available for reference concerning the specifics of BSC use, including a section on appropriate risk assessment. This document is available as Appendix A of the NIH/CDC manual Biosafety in Microbiological and Biomedical Laboratories (BMBL) (5th Edition).

VI.3.1.1. Class I BSCs

The Class I BSC provides personnel and environmental protection but no product protection. It is similar in airflow to a chemical fume hood, but has a High Efficiency Particulate Air (HEPA) filter in the exhaust system to protect the environment. In the Class I BSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as the minimum velocity of 75 linear feet per minute (lfpm) is maintained through the front opening. With the product protection provided by the Class II BSCs, general usage of Class I BSCs has declined. However, Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures (e.g., cage dumping, aerating cultures or homogenizing tissues) with a potential to generate aerosols.

The Class I BSC is hard-ducted to the building exhaust system, and the building exhaust fan provides the negative pressure to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the exhaust plenum. A second HEPA filter may be installed at the terminal end of the exhaust, prior to the exhaust fan.

VI.3.1.2. Class II (Types A1, A2, B1, and B2) BSCs

Class II BSCs provide personnel, environmental and product
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protection. Airflow is drawn around the operator into the front grille of the cabinet providing personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air has passed through the exhaust HEPA filter, it is contaminant-free (environment protection), and may be recirculated to the cabinet workspace, back into the laboratory (Class II Type A1 and A2 BSCs) or ducted out of the building (Class II Type B1, B2, and A2 BSCs). A Class II Type A2 BSC may optionally be installed such that air re-circulates back into the room or is ducted outdoors. Under the new NSF/ANSI 49 Standard, no newly installed Type A cabinet may be directly ducted to the building’s exhaust system; thimble exhaust connections should be used to connect all new installations of Type A BSCs to the building exhaust system.

HEPA filters are effective at trapping particulates and infectious agents, but not at capturing volatile chemicals or gases. Only Class II Type B2 BSCs that have 100% of air ducted to the outside should be used when working with volatile chemicals. Class II Type B1 BSCs recirculate 30% of exhaust air to the work area and should ONLY be used with minute amounts of volatile chemicals as long as the re-circulating vapors do not present a problem in the work. The same is true of Class II Type A2 cabinets that are vented to the outdoors (with the exception that this type of cabinet re-circulates 70% of the air back to the cabinet); when the Type A2 cabinet is vented back into the room, it should NOT be used with toxic chemicals.

All Class II cabinets are designed for work with microorganisms assigned to Risk Groups 1, 2 and 3. Class II cabinets provide the microbe-free work environment necessary for cell culture propagation, and also may be used for the formulation of nonvolatile antineoplastics or chemotherapeutic drugs.

VI.3.1.3. Class III BSCs

Class III cabinets provide the highest level of protection. A Class III BSC is a totally enclosed glove-box cabinet of gas-tight construction. The cabinet is maintained under negative air pressure of at least 0.5 inches of water gauge. Supply air is drawn into the cabinet through HEPA filters, and the exhaust air is filtered by two HEPA filters in series
before discharge to the outside. Generally, the ventilation system is separate from the facility’s ventilation system. Class III cabinets are available for high-risk biological agents.

**VI.3.1.4. Horizontal Laminar Flow “Clean Benches”**

Horizontal Laminar Flow “Clean Benches” are **not** BSCs. HEPA-filtered air flows across the work surface and toward the user. These devices provide product protection ONLY. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. These benches should not be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials. The worker is exposed to materials (including proteinaceous antigens) being manipulated on the clean bench and can experience hypersensitivity reactions. Horizontal clean air benches should never be used as a substitute for a biological safety cabinet in research, biomedical or veterinary laboratories or as a substitute for a chemical hood.

**VI.3.1.5. Vertical Laminar Flow “Clean Benches”**

Vertical Laminar Flow “Clean Benches” are also **not** BSCs. They may be useful in hospital pharmacies when a clean area is needed for preparation of intravenous drugs. While these units usually have a sash, the air is discharged into the room under the sash, resulting in the same potential problems as the horizontal laminar flow clean benches.

**VI.3.1.6 Biological Safety Cabinets vs. Chemical Fume Hoods**

Biological Safety Cabinets (BSCs) and Chemical Fume Hoods (CFHs) are not interchangeable. BSCs and CFHs are different equipment designed for different applications. BSCs are for working with biological materials and CFHs are for use when working with hazardous chemicals.

There are two main differences between chemical fume hoods and biosafety cabinets. Chemical fume hoods have inward airflow, offering personnel protection only. Biological safety cabinets, on the other hand, have both inward and downward airflow, allowing for both
personnel and product protection (clean work environment). The second difference is that a chemical fume hood has no HEPA filtration on the exhaust offering no environmental protection, where a biological safety cabinet has HEPA filters on both the supply and the exhaust which provides both product and environmental protection.

It is important to understand the differences between these two types of equipment. If you have questions as to which type of equipment you should be using for your research, please contact Environmental Health and Safety for assistance.

VI.3.2. Use of Biological Safety Cabinets

Biological safety cabinets with the potential to be used to protect workers from hazardous biological agents shall be tested and certified after installation and before use, any time they are moved, when major repairs are performed and at least annually. According to NSF/ANSI Standard 49, prior to repair or replacement of components located in contaminated plenums, prior to relocation and in some cases prior to recertification, BSCs should be gas decontaminated by a qualified contractor. The PI shall provide annual certification records for each biosafety cabinet under that individual’s control. Testing shall meet the criteria in NSF/ANSI Standard 49 - 2012 Biosafety Cabinetry: Design, Construction, Performance, and Field Certification, Annex F. Call OEHS for information on the standard and a list of companies qualified to certify biological safety cabinets.

- A BSC is required in Biosafety Level 2 laboratories whenever a laboratory procedure results in the formation of an aerosol (below are a list of activities that are prone to aerosol formation);
  - Centrifugation
  - Vigorous shaking and mixing
  - Pipetting
  - Grinding
  - Aspiration/washing
  - Injection
  - Sonication
  - Working with materials under pressure
- A BSC is required for all pathogen manipulations performed
In a Biosafety Level 3 laboratory;

- **Biological safety cabinets are only effective when personnel operate them properly.**
  - Understand the function and use of the biological safety cabinet before beginning work;
  - Demonstrate proficiency in working in the BSC;
  - **No modifications may be made to any BSC without first contacting the Institutional Biosafety Officer.**

Open flames are not permitted to be used in BSCs. The flame creates turbulence which disrupts the pattern of HEPA-filtered air being supplied to the work surface. In addition, the heat from the continuous flame may damage the supply and/or exhaust HEPA filters, requiring replacement of the filters.

Biosafety cabinets are designed for a single operator. Never work with two or more people at a time in **any** BSC, regardless of manufacturer, model or size. Multiple users will cause air disruptions and potentially destroy the containment capabilities of the BSC, possibly creating personnel, product or environmental protection issues.

Any procedure specific exemption or waiver from this policy must be submitted to the Institutional Biosafety Officer and/or the Institutional Biosafety Committee Chair for review and approval prior to commencement.

A thorough evaluation of the proposed work (including the biological and chemical agents to be used and the procedures to be performed) must be executed before selecting the appropriate biological safety cabinet. Contact the Institutional Biosafety Officer for assistance when selecting a new biosafety cabinet.

Additional information on BSCs can be found in the “Biological Safety Cabinets” online training available on the OEHS training website.
VI.3.3. Other Safety Equipment

Leak proof containers for the processing, transporting or storage of etiologic agents are also safety equipment. An example of a leak proof container is the safety centrifuge cup/rotor that is designed to prevent the release of aerosols during centrifugation.

Personal protective equipment (PPE) (e.g., gloves, coats, gowns, shoe covers, boots, respirators, face shields, and safety glasses or goggles) is clothing and equipment generally used in combination with BSCs and other devices to contain the agents, animals, or materials during manipulation. PPE is covered in more detail in Chapter VII of this manual.

In situations where it is impractical to work in BSCs, personal protective devices may form the primary barrier between personnel and the infectious materials. Examples of such situations include certain animal studies, animal necropsy, and activities relating to maintenance, service, or support of the laboratory facility.

Appropriate safety equipment must be considered when performing a risk assessment for a particular project. The Institutional Biosafety Officer, OEHS, and/or the IBC must be consulted when additional containment devices are determined to be necessary.

○ **WARNING:** Chemical fume hoods and laminar-flow clean-air benches (both vertical and horizontal) are not to be used for work with biohazard materials.

VI.4. Facility Design (Secondary Barriers)

Secondary barriers not only protect the environment within the facility, but also outside the laboratory (and the community outside the facility) from exposure to infectious materials. The design of the facility provides the secondary barrier. The three facility designs are the basic laboratory, the containment laboratory, and the maximum containment laboratory.

Laboratories at the University should be inspected annually by OEHS
staff and found to be in compliance with the appropriate biosafety-level containment for the biohazards in use as defined by the NIH/CDC and University guidelines.

**Work with agents classified as RG 3 or Biosafety Level 3 must be approved by the IBC before being initiated.**

**VI.4.1. The Basic Laboratory**

The Basic Laboratory provides general space where work is done with viable agents that are not associated with disease in healthy adults; it may include Biosafety Levels 1 and 2 facilities. This laboratory is also appropriate for work with infectious agents or potentially infectious materials when the hazard levels are low and laboratory personnel can be adequately protected by standard laboratory practice. While work is commonly conducted on the open bench, certain operations are confined to BSCs (especially those that produce aerosols). Conventional laboratory designs are adequate.

**VI.4.2. The Containment Laboratory**

The Containment Laboratory has specialized engineering features that enable laboratory workers to handle hazardous materials without endangering themselves, the community, or the environment. The containment laboratory is described as a Biosafety Level 3 facility. The features that distinguish this laboratory from the basic laboratory are the provisions for access control and a specialized ventilation system. In all cases, a controlled access zone separates the laboratory from areas open to the public.

**VI.4.3. The Maximum Containment Laboratory**

The Maximum Containment Laboratory has special engineering and containment features that allow laboratory workers to safely conduct activities involving infectious agents that are extremely hazardous to humans or capable of causing serious epidemic disease. The maximum containment laboratory is described as a Biosafety Level 4 facility. Containment requirements at this level will not be approved at the University.
VI.5. Recombinant and Synthetic Nucleic Acid Biosafety Levels

Laboratory-scale recombinant and synthetic nucleic acid research and development (i.e., <10 liters) must be carried out at the biosafety level determined to be appropriate by review of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. Although some experiments are found to be exempt from IBC review under the NIH Guidelines for purposes of genetic engineering, the containment necessary for performing these experiments is dependent upon the biosafety level assigned to the host/vector system.

Large-scale recombinant and synthetic nucleic acid production (≥ 10 liters) must be approved by the IBC. The appropriate level of containment will be determined by the IBC at the time of review of the protocol.