

BIOSAFETY CONTAINMENT LEVELS

(For assistance, please contact EHS at (402) 472-4925, or visit our web site at <http://ehs.unl.edu/>)

Purpose

The purpose of this SOP is to summarize the various levels of biocontainment established under the following standards:

- *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, Centers for Disease Control and National Institutes of Health¹
- *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*, National Institutes of Health²

Scope

All work that is subject to the UNL Biosafety Guidelines must be conducted under the appropriate level of containment. The Principal Investigator (PI) is required to specify the proposed level of containment in the written protocol that is submitted to the Institutional Biosafety Committee (IBC) for review. The IBC is responsible for setting the final containment level as part of the protocol review and approval process. The standard containment recommendations for laboratory practices/procedures, safety equipment, or facility features may be modified for a specific protocol as supported by the risk assessment. Deviations or enhancements to the standard containment conditions must be specified.

Overview of Containment Levels

Following is a list of containment categories and levels established by the BMBL and/or NIH Guidelines. The appropriate level of containment is selected based on the type of proposed work and activities and protocol-specific risk assessment.

| Standard microbiological and rDNA <i>in vitro/in vivo</i> , & cell/tissue culture work | Large scale (> 10 L of culture) | Large research animals | Whole plants-Greenhouses |
|--|-----------------------------------|------------------------|--------------------------|
| BSL- 1 | Good Large Scale Practices (GLSP) | ABSL-1 | BL-1-P |
| BSL- 2 | BL-1 (Large Scale) | ABSL-2 | BL-2-P |
| BSL- 3 | BL- 2 (Large Scale) | ABSL-3 | BL-3-P |
| BSL- 4 | BL- 3 (Large Scale) | ABSL-4 | BL-4-P |

Each of these containment levels is characterized by standard laboratory practices, safety equipment, and facility features, as summarized in tables 1 through 4. Refer to the NIH Guidelines and BMBL for a complete discussion on practices, primary barriers, safety equipment, and facilities.

TABLE 1
Standard microbiological and rDNA *in vitro/in vivo* and cell/tissue culture work

| BSL | PRACTICES | PRIMARY BARRIERS AND SAFETY EQUIPMENT | FACILITIES (SECONDARY BARRIERS) |
|------------|---|--|--|
| 1 | Standard Microbiological Practices | None required | Laboratory bench and sink required |
| 2 | BSL-1 practice plus: <ul style="list-style-type: none"> • Limited access • Biohazard warning signs • “Sharps” precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies | Primary barriers: <ul style="list-style-type: none"> • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials Personal Protective Equipment: <ul style="list-style-type: none"> • Laboratory coats; gloves; face protection as needed | BSL-1 facilities plus: <ul style="list-style-type: none"> • Autoclave available |
| 3 | BSL-2 practice 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 Personal Protective Equipment: <ul style="list-style-type: none"> • Protective laboratory clothing; gloves; respiratory protection as needed | BSL-2 facilities 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 | 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 facilities plus: <ul style="list-style-type: none"> • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements outlined in the text |

From BMBL 5th Ed.¹

TABLE 2
Large scale research or production of organisms containing rDNA
(> 10 L of culture)

| BL (LS) | PRACTICES | PRIMARY BARRIERS AND SAFETY EQUIPMENT | FACILITIES (SECONDARY BARRIERS) |
|-------------|--|--|--|
| GLSP | <ul style="list-style-type: none"> Standard Microbiological Practices Addition of materials to a system, sample collection, transfer of culture fluids and processing of culture fluids are done in a manner that minimizes the health risk to workers | Barriers: <ul style="list-style-type: none"> Systems designed to contain culture fluid and viable organisms PPE <ul style="list-style-type: none"> Provided as appropriate | <ul style="list-style-type: none"> Hand washing sink, shower, changing area available as appropriate |
| 1 | <u>GLSP facilities plus:</u> <ul style="list-style-type: none"> Spills and accidents reported immediately to the Lab Director; medical evaluation, surveillance and treatment provided as appropriate and recorded Minimization of aerosol formation when adding materials to the closed system or transferring between closed systems Emergency plans contain procedures for handling large losses of culture | <u>GLSP barriers and PPE plus:</u> <ul style="list-style-type: none"> Cultures of viable organisms handled in a closed system or other primary containment equip. (e.g., BSC with centrifuge for processing culture fluids) Culture fluids are not removed from containment unless the organisms have been inactivated Removal of fluid for analysis, further processing or final fill must be done by way of a closed system | <u>GLSP facilities plus:</u> <ul style="list-style-type: none"> HEPA filters in place to treat exhaust gases from closed systems or other primary containment equip. and prevent release of viable microorganisms containing rDNA Closed systems shall not be opened for maintenance unless the system has been sterilized and validated |
| 2 | <u>BL-1 Large Scale plus:</u> <ul style="list-style-type: none"> Spills and accidents reported immediately to the BSO, IBC, NIH/OBA, and other authorities; medical evaluation, surveillance and treatment provided as appropriate and recorded Closed systems are permanently identified and the identification used in all records Biohazard signs are posted on each closed system and primary containment equip. | <u>BL-1 Large Scale equipment plus:</u> <ul style="list-style-type: none"> Cultures of viable organisms handled in a closed system or other primary containment equip. (e.g., Class III BSC with centrifuge for processing culture fluids) Closed systems for propagation and growth shall have monitoring systems to verify containment integrity during operation | <u>BL-1 Large Scale plus:</u> <ul style="list-style-type: none"> Rotating seals and other mechanical devices associated with closed systems are designed to prevent leakage or enclosed in ventilated housings with HEPA filters |
| 3 | <u>BSL-2 Practices plus:</u> <ul style="list-style-type: none"> Specific entry and exit procedures and restrictions. | <u>BSL-2 primary barriers, equipment, and PPE plus:</u> <ul style="list-style-type: none"> Head space above culture in closed system is maintained at a pressure as low as possible to maintain containment integrity Closed systems are tested for integrity of containment with the host organism to be used; records are kept Garment change out required. | <u>BSL-2 Facilities Plus:</u> <ul style="list-style-type: none"> Controlled areas (double-doored space, separation from remaining facility, sealed penetrations, etc.) Hands free sink operation Shower Negative air flow with HEPA filtration on exhaust |

From Appendix K of the NIH *Guidelines*²

TABLE 3
SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR ACTIVITIES IN WHICH
EXPERIMENTALLY OR NATURALLY INFECTED VERTEBRATE ANIMALS ARE USED

| ABSL | PRACTICES | PRIMARY BARRIERS AND SAFETY EQUIPMENT | FACILITIES (SECONDARY BARRIERS) |
|----------|--|---|---|
| 1 | Standard animal care and management practices, including appropriate medical surveillance programs | As required for normal care of each species | Standard animal facility: <ul style="list-style-type: none"> • No recirculation of exhaust air • Directional air flow recommended • Hand washing sink is available |
| 2 | ABSL-1 practices plus: <ul style="list-style-type: none"> • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual • Decontamination of all infectious wastes and of animal cages prior to washing | ABSL-1 equipment plus primary barriers: <ul style="list-style-type: none"> • Containment equipment appropriate for animal species Personal Protective Equipment: <ul style="list-style-type: none"> • Laboratory coats, gloves, face and respiratory protection as needed | ABSL-1 plus: <ul style="list-style-type: none"> • Autoclave available • Hand washing sink available • Mechanical cage washer recommended |
| 3 | ABSL-2 practices plus: <ul style="list-style-type: none"> • Controlled access • Decontamination of clothing before laundering • Cages decontaminated before bedding removed • Disinfectant foot bath as needed | ABSL-2 equipment plus: <ul style="list-style-type: none"> • Containment equipment for housing animals and cage dumping activities • Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPEs: <ul style="list-style-type: none"> • Appropriate respiratory protection | ABSL-2 facility plus: <ul style="list-style-type: none"> • Physical separation from access corridors • Self-closing, double-door access • Sealed penetrations • Sealed windows • Autoclave available in facility |
| 4 | <div style="display: flex; align-items: center;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg); color: red; font-weight: bold; padding-right: 5px;">Not allowed at UNL</div> <div style="flex-grow: 1;"> ABSL-3 practices plus: <ul style="list-style-type: none"> • Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting • All wastes are decontaminated before removal from the facility </div> </div> | ABSL-3 equipment plus: <ul style="list-style-type: none"> • Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure personnel suit) used for all procedures and activities | ABSL-3 facility plus: <ul style="list-style-type: none"> • Separate building or isolated zone • Dedicated supply and exhaust, vacuum and decontamination systems • Other requirements outlined in the text |

From BMBL 5th Ed. ¹

Additional References:

Institute for Laboratory Animal Research. *Guide for the care and use of laboratory animals*. Washington, DC: National Academy Press; 1996.

National Institutes of Health, Office of Laboratory Animal Welfare. *Public Health Service policy on humane care and use of laboratory animals*, Bethesda (MD); The National Institutes of Health (US); 2000.

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Physical and Biological Containment for Plants, Microbes, and Insects

The principal purpose of plant containment is to avoid the unintentional transmission of an rDNA-containing plant genome, including nuclear or organelle hereditary material or release of rDNA-derived organisms associated with plants.

Generally, the organisms that are used pose no health threat to humans or higher animals, and the containment conditions are designed to minimize the possibility of an unanticipated detrimental effect on organisms and ecosystems outside of the experimental facility.

Practices and Physical Containment

These procedures and guidelines are summarized in Table 4 below.

Biological Containment

Procedures to prevent dissemination of: genetic material from experimental plants by pollen and seed; microorganisms; and insects (arthropods).

| Plants | Microbes | Insects |
|---|---|--|
| <ul style="list-style-type: none"> • Use genetic engineering techniques that localize transgenes to non-propagative plant parts or confer plant sterility • Cover or remove flower and seed heads to prevent pollen and seed dispersal • Use male sterile strains • Harvest the plant material prior to the reproductive stage • Control flowering time so pollen shed does not occur during the receptive period of nearby cross-fertile plants • Ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant | <ul style="list-style-type: none"> • Genetically disable the microbes to minimize survival and reproduction • Avoid creating aerosols when inoculating plants • Provide adequate distance between an infected plant and another susceptible host; especially if dissemination can occur through the air or by leaf contact • Grow experimental plants and microbes at a time of year when susceptible plants are not growing nearby • Eliminate vectors for insect-borne microbes • Choose microbes with an obligate association with the host plant • Treat runoff water to kill living organisms | <ul style="list-style-type: none"> • Choose or create non-flying, flight-impaired, or sterile strains • Conduct experiments at a time of year when escaped organisms will not survive • Choose organisms that do not have an obligate association with nearby plants • Treat or evaporate runoff water to eliminate viable larvae and eggs • Avoid use of small insects in greenhouse cages • Destroy all pollinating insects in cages after pollen transfer |

Additional References:

A Practical Guide to Containment: Plant Biosafety in Research Greenhouses, D. Adair & R. Irwin, Information Systems for Biotechnology; Blacksburg, VA; 2008. <http://www.isb.vt.edu/>

TABLE 4
SUMMARY OF GUIDELINES FOR BIOSAFETY CONTAINMENT LEVELS FOR PLANTS,
ARTHROPODS AND THEIR ASSOCIATED MICROBES IN GREENHOUSES^{1,2}

| BL-P | PRACTICES | FACILITIES (SECONDARY BARRIERS) |
|------|--|---|
| 1 | Standard BSL-1 Practices plus: <ul style="list-style-type: none"> • Personnel must read and follow written greenhouse practices and procedures • Experiments currently in progress are recorded • Inactivation of experimental organisms before disposal outside of greenhouse • Undesired species control plan implemented • Motile macroorganisms are housed in appropriate cages and if released, escape from the facility is minimized | <ul style="list-style-type: none"> • Greenhouse floor is composed of gravel or other porous material and walkways are of an impervious material (e.g. concrete) • Windows and other openings may be open and do not require barriers to contain or exclude pollen, microbes, or small flying animals • Screens are recommended |
| 2 | BL1-P practices plus: <ul style="list-style-type: none"> • Records of all plants, microbes or small animals brought in or removed from the facility • Any accidental spill or release of microbe shall be reported to the GD*, IBC, NIH/OBA and other applicable authorities • Decontamination of run-off water is recommended • Gravel or similar floors should be treated periodically to inactivate/eliminate potentially trapped organisms • Signs must be posted when a restricted experiment is in progress • Signs should be posted if organisms with potential for detrimental impact on managed or natural ecosystems and/or risk to human health are present • A greenhouse practices manual should be prepared and include contingencies for unintentional release of organisms | BL1-P facility plus: <ul style="list-style-type: none"> • Greenhouse floor is composed of an impervious material (e.g. concrete) • Screens on windows and openings to exclude birds and arthropods • Autoclave available • Minimize the ingress of arthropods through intake fans • Containment can be satisfied by using a growth chamber or growth room within a building that limits access and escape of micro and macroorganisms in a way that satisfies the intent of BL2-P guidelines |
| 3 | BL2-P practices plus: <ul style="list-style-type: none"> • Access restricted to those required for program or support purposes • Any accidental spill or release of microbe shall be reported to the BSO† in addition to those identified in BL2-P practices • Experimental materials are sterilized in an autoclave or rendered biologically inactive before disposal, including water • Decontamination of containers used to transport materials into or out of the facility • Disposable clothing is worn if deemed necessary by the GD; disposable clothing is removed before exit and decontaminated prior to washing or disposal • Hands are washed upon exiting the facility • All procedures are performed to minimize aerosol formation and excessive splashing of soil/potting material | BL2-P facility plus: <ul style="list-style-type: none"> • Standard BSL-3 facility design • Greenhouse floor is composed of an impervious material (e.g. concrete) with provision for collection and decontamination of liquid run-off • Windows are closed and sealed and resistant to breakage • Double door (i.e. pass-through) autoclave is recommended • Vacuum lines are protected with HEPA filters and liquid disinfectant traps |
| 4 | BL3-P practices plus: <ul style="list-style-type: none"> • Standard BSL-4 practices • Access is managed by the GD, BSO or other individual responsible for physical security of the greenhouse • All personnel entering and exiting the facility are recorded • All materials are autoclaved prior to removal from the facility • Undesired pests & pathogens are eliminated through a chemical control plan • Experimental materials are transported into and removed from the facility in primary and secondary non-breakable and sealed containers; an airlock, fumigation chamber or similar is used to remove these containers from the facility • Supplies and materials are brought into facility through an airlock, fumigation chamber or pass-through autoclave that is decontaminated between each use | BL3-P facilities plus: <ul style="list-style-type: none"> • Standard BSL-4 Facility Design, except: <ul style="list-style-type: none"> ◦ Class III BSC not required for work • Access doors are self-closing and locking • Backup power source for HVAC system • Sewer vents and other ventilation lines are HEPA filtered |

*GD – Greenhouse Director

†BSO – Biosafety Officer