

BL2+ Projects: Generic Requirements for Laboratory Facility and Biosafety Manual

Scope/Summary: This policy outlines the requirements by Principal Investigators (PIs) to conduct work at Biosafety Level 2+ (BL2+) at MIT.

Background: BL2+ containment is suitable for work with risk group 2 (RG2) organisms where modifications to the organism or changes to the process present an increased safety risk to researchers handling the material. Examples include use of a lentiviral vector to deliver an oncogene or knock down a tumor suppressor, work with multidrug resistant pathogens, aerosolization studies of a pathogen, or use of high concentrations or volumes of a pathogen. BL2+ is also suitable for work with specific RG3 agents, which may cause serious disease but are not transmitted by inhalation (i.e. Human Immunodeficiency Virus), or for some RG2 agents that can cause moderate to severe disease and are transmitted by inhalation.

I. General Policies and Procedures:

- Facility design, procedures and practices must fulfill all requirements of a BL2 laboratory with the addition of specific BL3 practices and procedures, as outlined in Biosafety in Microbiological and Biomedical Laboratoriesⁱ and the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Moleculesⁱⁱ.
- The suitability of BL2+ containment for a given research protocol is decided on a case-by-case basis by the Environment, Health and Safety Office (EHS) Biosafety Program (BSP), the MIT Committee on the Assessment of Biohazards and Embryonic Stem Cell Research Oversight (CAB/ESCRO), and the PI.
- Research conducted at BL2+ may not be given administrative approval by the MIT Institutional Biosafety Officer. Review and approval by the CAB/ESCRO is required before initiation. Change in personnel or addition of mammalian cell lines that have the same risk profile as already approved cell lines may be considered for administrative approval.
- The PI must indicate those individuals that will work at BL2+ so that all access policies, procedures, and training needs are clear and fulfilled (with documentation). Access is controlled by the PI and is restricted to those persons whose presence is required for experimental or support purposes.
- Undergraduate students may NOT handle BL2+ materials.
- If lower containment work is done in parallel with BL2+ research, all work must conform to the BL2+ practices and procedures.
- A universal biohazard sign with a BL2+ designation must be posted on the outside of the door to the laboratory. The warning sign identifies the infectious agent, lists the name and telephone number of the PI and other responsible person(s), and indicates the special requirements for entry into the laboratory

- All manipulations involving viable BL2+ materials must be conducted in a biological safety cabinet (BSC) or other physical containment devices within the laboratory. No work in open vessels/tubes/flasks/culture dishes should be conducted on the open bench unless the material has been completely lysed or otherwise inactivated using methods that are well known to be effective.
- The generation of aerosols must be minimized in experimental procedures.
- Gloves must be replaced often. Contaminated gloves must never be used to handle common equipment.
- Lab coats cannot be worn outside of BL2+, and must be decontaminated before removal from BL2+ laboratory. Disposable lab coats are recommended.
- Investigators are encouraged to cover paper notes with clear plastic covers when brought into the BL2+ lab.

II. Equipment:

- BL2+ laboratory equipment (including but not limited to automatic pipetters, micropipettes, microscopes, counting chambers, water baths, etc.) must be dedicated for use with BL2+ materials whenever possible. Equipment may not be brought out into general laboratory area for use in other projects without decontamination. This may not be possible for all equipment such as ultracentrifuges and liquid nitrogen storage.
- Procedures for the removal of BL2+ materials from the BL2+ laboratory and any use of equipment outside the BL2+ space must be outlined in the project-specific biosafety manual with attention given to prevention of possible contamination of equipment or spaces outside of the BL2+ laboratory.
- A BSC or other physical containment device is required. BSCs must be vented to the outside; exhaust air is not recirculated into laboratory. Exception to this must be approved by the CAB/ESCRO.
- Centrifuge buckets and carriers must have secondary safety buckets so that if tubes or plates leak all liquid is contained. Loading and unloading of all centrifuge bottles, carriers, and tubes must be done in the BSC. Outer surfaces must be wiped with approved disinfectant each time they are removed from the BSC.
- A hand washing sink must be present in a BL2+ lab. The sink should be hands-free (e.g. foot operated or infrared sensor), or have paddle faucet handles. If possible, a small leak detector is placed under the sink so that repairs can occur prior to a large-scale leak.
- An emergency eyewash must be present.
- Laboratory chairs must not have cloth covers. All laboratory surfaces should be easily cleaned and decontaminated with appropriate disinfectants.
- A spill clean-up kit, sufficient to deal with the largest volumes used in experiments, must be present. Researchers working in the BL2+ laboratory should know how to deal with a

spill of materials and use the spill cleanup kit. This should be part of the facility orientation program for people working in the BL2+ space.

- Vacuum lines must have an inline HEPA filter. Suction flasks should have disinfectant present and have an overflow flask with the HEPA filter in place between the overflow flask and the house vacuum line or pump. The objective is to prevent contamination of building vacuum lines or pump. Suction and overflow flasks should be in a secondary container to prevent breaking and contain liquid should leak occur.
- There is no requirement for an autoclave within a BL2+ laboratory; however, one must be close by for waste decontamination unless other provisions are made for proper waste disposal.
- Cardboard biowaste boxes are not to be stored in the BL2+ laboratory (due to difficulty in properly decontaminating porous cardboard). Boxes should be stored in the anteroom or outside the BL2+ laboratory but always within a biological laboratory. Only plastic or metal biowaste bins are used within the BL2+ laboratory.

III. Facility Design:

- For new BL2+ space renovations, the laboratory plan must meet all appropriate building and safety codes. Renovation plans should be reviewed and approved by BSP before renovations begin. Unique laboratory designs may require CAB/ESCRO review.
- A door must physically separate the BL2+ laboratory from other research and public areas. The door must be kept closed at all time with access by special key or key card. While BL2+ materials are present in the facility (either stored in freezers or refrigerators or in incubators), the door must be locked when no one is present. The entry door should be self-closing. The door should have a window so that personnel working at BL2+ can be seen (safety issue).
- BL2+ laboratory space must have directional airflow such that air flows from relatively uncontaminated public spaces and/or lower biosafety containment areas into the BL2+ laboratory.
- A sign indicating the location of shutoff valves for gas, water, vacuum, and electricity to the BL2+ facility must be posted on the entry door to the facility so that services may be shutdown as needed without having to enter the research laboratory.
- Consideration should be given to storing CO₂ tanks outside the BL2+ facility for easier replacement.
- The finished BL2+ facility must be inspected by BSP prior to start of work and at least annually after commissioning.

IV. Written Procedures:

- A project-specific BL2+ biosafety manual must be developed by the PI and approved by the MIT Institutional Biosafety Officer and the CAB/ESCRO. The manual will be reviewed and updated as needed and at least annually.

- The project-specific BL2+ biosafety manual must contain documentation of the following:
 - Written laboratory work procedures, including the donning, doffing, and general use of personal protective equipment (PPE).
 - All waste handling and decontamination procedures.
 - An outline of the Training Program to be used for researchers planning to work at BL2+.
 - Procedures for the removal of BL2+ materials from the BL2+ laboratory, and any use of equipment outside the BL2+ space must be noted and the measures to be taken to prevent possible exposures clearly outlined.
 - Storage locations for BL2+ materials such as viral stocks must be clearly identified.
 - Procedures to be followed in case of an exposure, spill, and spill clean up.
 - Any requirement for medical monitoring, immunization, or serum samples must be outlined and documentation of medical consultation and compliance provided.

V. Training:

- Training for ALL personnel entering the BL2+ laboratory will be conducted. Training will cover this policy, the project-specific BL2+ biosafety manual, and any standard operating procedures (SOPs) developed in conjunction with these BL2+ requirements. BSP will review these SOPs.
- The PI is responsible for insuring that, prior to working with organisms at BL2+ containment, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This may include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the PI or other competent scientist proficient in safe microbiological practices and techniques.
- Laboratory personnel must receive appropriate training on the potential hazards associated with the work, the necessary precautions taken to prevent exposures, and exposure evaluation procedures.
- Personnel receive annual updates or additional training as necessary for procedural or policy changes.
- Trained personnel will sign an attendance sheet, a copy of which will be maintained in the BSP Biological Research Registration file, and with the PI.
- Required Training Courses:
 - **Course 220s: Signature: I have read Lab's BL2+ manual**
 - **Course 221c: BL2+ Training**
 - **Course 257c: Biosafety Cabinet Training**
 - **Course 230c: Lab-specific Pathogen Training**

References:

- ⁱ [CDC/NIH, Biosafety in Microbiological and Biomedical Laboratories.](#)
- ⁱⁱ [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.](#)

Revision History:

Approved 09/2005
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