



Biosafety Fact Sheet



BASICS OF BIOSAFETY LEVEL 2 WITH STIPULATIONS

BASICS OF BIOSAFETY LEVEL 2+ (BL2+)

Biosafety Level 2 with stipulations refers to a Basic Laboratory with biosafety cabinets (BSC) and other physical containment devices, utilizing BL3 practices and procedures. Examples of BL2+ work include the use of lentiviruses that are not routinely tested for replication-incompetence and for production of stocks of HIV-1 and SIV (retroviruses), Semliki Forest Virus, and His-TAT-NLS-Cre (an oncogenic and potentially toxic vector).

Exposure risks at BL2+ include:

- Accidental needle sticks or cuts;
- Exposure to the mucous membranes of the eyes, nose and mouth; or
- Ingestion of infectious materials (due to failure to wash hands after handling infectious materials and before leaving the laboratory).
- Laboratory procedures with aerosol or high splash potential.

The Following Standard Microbiological Practices Must Be Followed in BL2+ Laboratories

- Staff must be trained on procedures specific to the work being performed in the laboratory.
- Training in the OSHA Bloodborne Pathogens Standard and Universal Precautions procedures must be given before work begins and must be updated annually. Contact the Harvard Institutes of Medicine/New Research Building (HIM/NRB) Environmental Health and Safety (EH&S) Office if staff needs refresher training.
- A BL2+ Biosafety Manual must be written, updated annually, and read by all BL2+ personnel before being authorized to work in the BL2+ laboratory.
- Only Authorized Personnel are permitted to work in the BL2+. The Principal Investigator controlling the BL2+ lab authorizes personnel only after they have completed BL2+ safety training (from the HIM/NRB EH&S Office) and job-specific training from the PI or designated employee.
- No immunocompromised persons allowed in the BL2+ lab.
- The Principal Investigator (PI), along with Occupational Health, needs to determine if immunizations are required based on the agents being used.
- No custodial or maintenance personnel permitted to enter without authorization of the PI.
- Laboratory door is locked at all times.
- Post biohazard warning sign on the door indicating the infectious agent(s), name and telephone numbers of the PI and other responsible persons, and special requirements for entering the room (such as vaccinations and personal protective equipment [PPE]).
- Appropriate PPE must be worn in BL2+ laboratories, including disposable, solid-front gown, 2 pairs of disposable latex or nitrile glove, safety glasses, and a face shield when splashes are possible.
- Do not wear PPE outside of the BL2+.
- Cover all cuts and abrasions with bandages before putting on disposable gloves.
- Disposable gloves should be changed frequently and hands should be washed between glove changes.
- Skin care should be a primary concern: lotions and creams should be used to prevent chapping and dermatitis (be sure to use lotions and creams that do not degrade latex: order from a commercial vendor such as Fisher Scientific).
- Wash hands before leaving the laboratory.
- No eating, drinking, chewing gum, smoking, or applying cosmetics is allowed in the BL2+ laboratory at any time. This is to prevent ingestion and absorption of infectious materials, chemicals, or radionuclides.
- No flowers or plants allowed, unless they are related to the work.
- No mouth pipetting is permitted.
- Sharps must be disposed in special Sharps Containers. Sharps include needles, scalpel blades, and Pasteur pipettes. Do not fill Sharps Containers more than 2/3 full.



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- Decontaminate work surfaces daily, at a minimum. Decontaminate the work surface inside the BSC with 70% Ethanol before and after use.
- Decontaminate the plenum (area under the work surface of the BSC) at least weekly.
- If aspiration flasks are outside of a BSC, they must be in secondary containment to prevent spills on countertops and floors.
- In-line HEPA or HEPA-like filters must be used for all aspiration flasks.
- Universal Precautions procedures must be followed: consider all human blood, body fluids, tissues, and primary human cell lines where the presence of an infectious agent may be unknown to be infectious at all times. Never handle these samples on an open bench; handle only in a Class II biosafety cabinet, while wearing a disposable, solid front gown and disposable latex or nitrile gloves.
- All procedures with infectious materials must be performed inside a Class II biosafety cabinet that is certified annually. No work with open vessels if infectious materials on open bench.
- Lab equipment and work surfaces routinely disinfected with suitable disinfectant (after completing work with infectious materials and after spills, splashes, or other contamination with infectious materials).
- Suitable disinfectants are vesphene II (2%-5%), bleach (10%, made weekly), and ethanol (70%).
- Use disposable plasticware. Nothing goes to Glasswash from BL2+ rooms.
- At the end of each day, bench tops must be wiped down with a suitable disinfectant.
- All infectious materials for centrifugation must be placed into aerosol containment canisters (opened and closed inside BSC). Inspect O-rings and canisters periodically to ensure proper function.
- When 1/2 full, vacuum flask, rubber stopper, tubing, and side-arm must all be covered with aluminum foil, sealed with autoclave tape, and autoclaved at 121 °C for 30 minutes for liquids, prior to disposal.
- Once/week, beakers/buckets for pipettes must be covered with aluminum foil, sealed with autoclave tape, and autoclaved at 121 °C for 1 hour for solids.
- Pipettes, after soaking in a beaker/bucket of disinfectant for 30 minutes, must be placed into boilers, sealed with autoclave tape, and autoclaved at 121 °C for 1 hour prior to disposal.
- Prior to disposal, a working concentration of disinfectant is added to tissue culture flasks (inside the BSC). Flasks are then placed into autoclavable buckets containing a suitable disinfectant (such as 2% Vesphene II). When buckets are ½ full, autoclave at 121 °C for 30 minutes for liquids. Pour contents of autoclaved liquid down sink, and discard autoclaved flask in biohazard waste container.
- All solid wastes (paper towels, disposable gowns, gloves, etc) are autoclaved at 121 °C for 1 hour prior to disposal in biohazard waste container.
- Autoclave validation must be performed regularly: chemical tests with temperature sensitive tape at every run and biological indicator tests every 2 weeks. Autoclave Validation Log must be posted for viewing.
- If Kill Stations are under sinks, routine maintenance must be performed, such as ensuring the tip chute is not blocked.
- Ensure staff knows how to clean/decontaminate spills in centrifuges, incubators, biosafety cabinets, and in the laboratory (both minor and major spills).
- Use secondary containment whenever transporting BL2+ materials outside of the laboratory. This can be a plastic container, as long as the cover will not fall off if the container is dropped. Spray the outside of the secondary container with a suitable disinfectant before removal from the laboratory.
- Follow the one-glove rule: wear a glove on the hand carrying the secondary container. Wear no gloves on the hand you'll use to open doors, etc.
- Report injuries and possible exposures immediately to the Laboratory Administrator or Department Administrator. Seek medical attention for injuries requiring more than basic First Aid. Submit Injury Form to the HIM/NRB EH&S Office within 2 working days.
- Report illnesses suspected to be work-related to the Laboratory Administrator or Department Administrator and to the Safety Office and Occupational Health immediately.
- Ensure staff knows what to do in the event of a Fire Alarm or Evacuation Order.