

Enhanced BSL-2 (BSL-2+)

All lab members must review this policy and sign/date the confirmation page at the end.

I. GENERAL INFORMATION

- i. Institutional Oversight: Work requiring Enhanced BSL-2 (BSL-2+) practices must be reviewed and approved by the Dartmouth Institutional Biosafety Committee (IBC) prior to commencement.
- ii. Biosafety Procedures: The lab will keep a copy of this policy and applicable lab safety manuals accessible to all lab members. The lab will comply with all applicable IBC and EHS policies and procedures in the conduct of Enhanced BSL-2 research. IBC and EHS policies, guides, manuals, and standard operating procedures may be found on the Biosafety Program website: http://www.dartmouth.edu/~ehs/biological/policies_sops.html.

II. RESPONSIBILITIES

A. Principal Investigator (PI)

- i. Complete all applicable portions of their BioRAFT biological registration as they pertain to the proposed Enhanced BSL-2 research.
- ii. Perform initial risk assessment of work with assistance from the Biosafety Officer (BSO).
- iii. Obtain IBC approval for work outlined in BioRAFT.
- iv. Ensure that all lab personnel are properly trained in BSL-2 practices and procedures and in the specific Enhanced BSL-2 practices described in BioRAFT.
- v. Comply with the [NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules](#) (NIH Guidelines) and [Biosafety in Microbiological and Biomedical Laboratories](#) (BMBL).

B. Dartmouth IBC

- i. Determine whether research requires Enhanced BSL-2 practices based on a review of submitted research, the initial risk assessment of proposed work, and engineering controls available in the research facility.
- ii. Review and approve research requiring Enhanced BSL-2 practices.
- iii. Ensure compliance with the *NIH Guidelines* and BMBL.



C. Biological Safety Officer

- i. Assist PI in risk assessment determination of work
- ii. Inspect research areas and report findings to the IBC
- iii. Review training compliance

D. All personnel conducting Enhanced BSL-2 work will

- i. Recognize the risks inherent in working with human pathogens and/or toxins, and agree to take responsibility for adhering to appropriate protective measures as outlined in institutional guidelines and in this policy.
- ii. Have a working knowledge of the Enhanced BSL-2 procedures they will be performing, including what strains, biotoxins, or viral vectors that will be used.
- iii. Understand the location and proper use of biological safety cabinets, freezers, incubators, autoclaves, and other equipment related to the Enhanced BSL-2 research.
- iv. Restrict access to areas of the lab designated for Enhanced BSL-2 research so that only those who have read and signed this policy are permitted to enter. Unauthorized individuals may not enter the area when Enhanced BSL-2 work is underway and signage should be posted accordingly.

III. OCCUPATIONAL HEALTH

- i. Personnel Considerations: Personnel conducting Enhanced BSL-2 research should acknowledge all possible modes of exposure/transmission for the agent(s) they will be working with. They should also know in advance of work the signs or symptoms of possible exposure to the agents(s) and what current treatment(s) are available.
- ii. Immunizations: It is recommended that those working with human source materials receive the Hepatitis B vaccination. This will be provided free of charge. All laboratory personnel, whether they have direct or indirect contact with Enhanced BSL-2 agents, should consult with EHS and Occupational Medicine regarding possible vaccinations.
- iii. Incident (Exposure, Potential Exposure, or Spill) Response: Please refer to the Dartmouth [Emergency Response and Biohazard Exposure Control Plan](#) for details on spill clean up, exposure response procedures, medical surveillance, and reporting procedures.
 - All accidents and spills must be reported immediately to the Principal Investigator and/or lab supervisor.
 - In case of a splash into an eye, nose, or mouth, immediately flush the affected area for 15 minutes at the nearest eyewash station. In case of skin exposure, thoroughly wash the affected area with disinfectant soap and water for 10 minutes. In case of parenteral injection (e.g., puncture), forced bleeding is first recommended followed by thorough washing.
 - After administering first aid, injured personnel must report for immediate medical evaluation at Occupational Medicine (DHMC, 603-650-3850) between the hours of 7:30am-4:30pm.



Students report to Dick's House (Hanover campus, 603-646-9400) between 7:30am-4:30pm. Report to the DHMC Emergency Room after hours or on weekends. If transport assistance is needed, contact Safety and Security at 603-646-3333 (or 5555 if at DHMC). If exposure requires emergency treatment, call 911.

- The PI must report the incident to the Biosafety Officer (biosafety@dartmouth.edu) by emailing an electronic Biohazard Incident Report within 24 hours. If an injury was sustained, an Accident Report Form will need to be completed for the Office of Risk Management. The incident will be reviewed by the Institutional Biosafety Committee (IBC).

IV. ENHANCED BSL-2 PRACTICES and PROCEDURES

In addition to complying with standard BSL-2+ practices, as outlined in the BMBL and according to Dartmouth IBC and EHS policies, the following additional practices and procedures must be conducted when working at Enhanced BSL-2.

A. Disinfection/Deactivation:

Researchers are responsible for decontaminating solid and liquid Enhanced BSL-2 waste immediately upon the completion of each experiment. Solid waste must be immediately autoclaved, following Dartmouth autoclave procedures. Some biotoxins are not deactivated by autoclaving. Liquid waste must be disinfected/deactivated with appropriate methods for the agent studied.

B. Personal Protective Equipment

i. Gowns

- Back closing disposable gowns must be donned while working with Enhanced BSL-2 materials.
- All disposable lab gowns must be disposed of with Enhanced BSL-2 waste.
- Disposable gowns and soiled lab coats must not be worn outside of the lab.
- Soiled lab coats should be decontaminated (i.e. soaked in disinfectant or autoclaved) prior to laundering.

ii. Gloves

- Two pairs of gloves must be donned while working with Enhanced BSL-2 materials. The outside glove must be removed before leaving the biosafety cabinet and disposed of in the biohazard bag inside the cabinet. The inside glove is then removed outside of the cabinet and disposed of as biohazardous waste.
- Gloves must be disposed of when contaminated, replaced frequently, and removed when work with potentially infectious materials is completed or when leaving the area.
- Gloves must not be reused. Do not spray with alcohol to “wash”.
- Gloves must be removed before handling common non-lab equipment such as phones, desks, and door knobs.



- Gloves that fit over the sleeves of the lab gown are recommended.
 - Hands must be washed after removing PPE
- iii. Face protection
- Face protection (glasses or a faceshield) must be used whenever splashes or sprays of infectious or other hazardous materials may be generated and contamination to the face (eye, nose, or mouth) can be anticipated.

C. Manipulations of Enhanced BSL-2 Agents

- i. All manipulations of Enhanced BSL-2 materials must be performed in a properly maintained and certified biological safety cabinet (BSC).
- ii. No Enhanced BSL-2 work will be conducted on the open bench top. This includes opening containers of potentially infectious materials, pipetting, vortexing, transfer operations, plating, grinding, blending, drying, sonicating, etc.
- iii. No sharps are to be used when conducting Enhanced BSL-2 work if this is experimentally avoidable. Never recap, bend or break sharps.

D. Biosafety Cabinet (BSC) Use

- i. A beaker or container containing fresh 1:10 dilution of bleach or 0.5% Wescodyne should be used inside the biosafety cabinet to collect/disinfect pipettes and pipet tips prior to removal from the BSC. Allow a minimum of 20 minutes contact time before removal from the BSC.
- ii. Use a small, orange biohazard bag-lined waste container to collect solid biohazard waste (Eppendorf tubes, gloves, etc.) inside the BSC.
- iii. A biohazard sharps container should be available to collect sharps inside the BSC.
- iv. Appropriate disinfectant should be kept at each BSC for decontamination of work surfaces before and after each work session.
- v. All biohazardous materials should be transferred out from the biosafety cabinet in a leakproof container that has been tightly closed and decontaminated by spraying the outside with 70% ethanol, a 1:10 dilution of bleach, or other appropriate disinfectant.

E. Centrifugation

- i. Screw capped tubes and aerosol-containing canisters must be used (safety cups) or capped centrifuge buckets.
- ii. The outside surfaces of the tubes must be wiped down with 70% ethanol before placing into safety canisters or buckets.
- iii. After centrifugation, open the safety canisters or buckets inside the BSC.



- iv. If there has been any possibility of leakage during centrifugation, the inner walls of the centrifuge and the rotor must be immediately decontaminated. Leakages during centrifugation must be reported immediately to the Principal Investigator.

F. Water baths

- i. Enhanced BSL-2 materials can be thawed in the water bath if they are kept in a sealed and watertight container.

G. Vacuum Lines and Traps

- i. Lines must be protected with secondary traps and in-line hydrophobic filters.
- ii. Traps must contain either fresh 1:10 bleach or 0.5% Wescodyne solution (final concentrations).
- iii. Liquid trap waste should be decontaminated for a minimum of 20 minutes before drain disposal with excess water.
- iv. Filters should be replaced every 6 months or when they become clogged.

H. Pipette Aids

- i. When Enhanced BSL-2 material is pipetted using disposable pipettes in the biosafety cabinet, care should be taken to avoid aerosolization.

I. Pipettes and Pipette Tips

- i. Avoid generating aerosols when pipetting.
- ii. Decontaminate stripette pipettes by pipetting fresh 1:10 bleach waste solution up and down in the prior to autoclaving as biohazardous waste.
- iii. Pipette tips should be soaked in the bleach solution in the biosafety cabinet for at least 20 minutes.
- iv. After soaking, the pipettes should be carefully drained in the sink with excess water before disposal as autoclave waste.

J. Laboratory Doors

- i. The door must be self-closing and kept closed at all times during experimentation.
- ii. Doors should be locked when laboratory is unattended.
- iii. Doors must have an EHS sign identifying the hazards in the lab, including the universal biohazard symbol, and emergency contact info.



K. Labeling

- i. Equipment used for Enhanced BSL-2 work must be labeled with the universal biohazard symbol and marked “Enhanced BSL-2” or “BSL-2+”.
- ii. Labeling should include name, date, and the identity of the material.

L. Transport of ENHANCED BSL-2 material:

- i. The transport of Enhanced BSL-2 material between laboratory spaces will be done only in sealed, biohazard labeled containers such as a Tupperware box.
- ii. All containers used for transport must be clearly labeled with name, date and identity of the material.
- iii. Enhanced BSL-2 materials may not be transported to another lab or research area that is not registered and approved for Enhanced BSL-2 work. Any exceptions require EHS and/or IBC approval.
- iv. Enhanced BSL-2 material shall not be transported by personal vehicle. Packing and shipping requires DOT/IATA training. Contact biosafety@dartmouth.edu for more information.

M. Housekeeping

- i. When Enhanced BSL-2 work is in progress, custodial and maintenance staff will not be given access to the immediate areas in which the experiment is being conducted. Otherwise, service and maintenance staff should conduct themselves according to institutional policy.

V. RESOURCES

- *Biosafety in Microbiological and Biomedical Laboratories*, 6th ed. Centers for Disease Control and Prevention and National Institutes of Health, 2020 June
<https://www.cdc.gov/labs/BMBL.html>
- Emergency Response and Biohazard Exposure Control Plan. Dartmouth IBC 2022
http://www.dartmouth.edu/~ehs/biological/policies_sops.html
- *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*. National Institutes of Health Office of Science Policy, 84 FR 17858, 2019 April
<https://osp.od.nih.gov/biotechnology/nih-guidelines/>

