

**MANUAL FOR RESEARCH CONDUCTED UNDER**

**ENHANCED BSL2 CONDITIONS**

Insert Research Laboratory

Insert Location

**Date:\_\_\_\_\_\_\_\_\_\_Signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**(Primary Investigator)**

**Date:\_\_\_\_\_\_\_\_\_\_Signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**(Brian J. O’Shea, Ph.D., Biological Safety Officer)**

The primary investigator or responsible persons for the laboratory in which ENHANCED BSL2 research will be conducted shall sign the completed manual and return to the biological safety officer. This manual is meant as a guide and risk assessment for hazardous research and a copy shall be made available to all applicable personnel in the laboratory in question.

**SAFETY PRACTICES DURING ENHANCED BIOLOGICAL SAFETY LEVEL TWO (ENHANCED BSL2)**

Insert Location

Insert Research Laboratory

**A. OVERVIEW**

*Please give a detailed overview of the research project. Include purpose for research, strains used, reason for Enhanced BSL2 status, and time frame of research project.*

Insert Text Here

**B. LOCATION**

*Please describe the location where the Enhanced BSL2 research will be performed. Please include information pertaining to biological safety cabinets, freezers, incubators, autoclaves, and any other equipment related to Enhanced BSL2 research.*

Insert Text Here

**C. ACCESS RESTRICTIONS**

Access to areas of the lab being currently used for ENHANCED BSL2 research is restricted to lab members with ENHANCED BSL2 training. No glasswasher will handle ENHANCED BSL2 waste. No glasswasher, custodian, or maintenance staff will be allowed to interact with the biosafety cabinet or researcher at the biosafety cabinet when ENHANCED BSL2 work is underway.

**D. ADMINISTRATION**

1. **ENHANCED BSL2 Facility Supervisors**:

Insert Text Here

Brian O’Shea, Ph.D. / Biological Safety Officer / 646-9790

*Please list researcher supervisors (PI and lab manager) with contact information for each.*

**2. Institutional oversight and approval:** Work involving Enhanced BSL2 practices requires previous approval through the Institutional Biosafety Committee (IBC) before commencement of research.

**3. Written Biosafety Procedures**: The laboratory keeps documents describing the safe lab practices and procedures, developed in consultation with the EH&S office and IBC policies. All personnel involved in research in this lab are required to adhere to these written rules. These documents can be found on the Dartmouth EH&S website for Biological Safety. A copy of this document is to be kept for reference in the ENHANCED BSL2 laboratory in 314W as well as with the EH&S Office.

**4. Responsibilities**: The Dartmouth EH&S Office, on behalf of the IBC, has the right and responsibility to review, inspect and approve the use of the location mentioned above for ENHANCED BSL2 work as described in this document.

The Principal Investigator is responsible for ensuring that before beginning ENHANCED BSL2 work, all personnel demonstrate proficiency in standard microbiological practices and receive appropriate training on the potential hazards associated with the work involved and the operations specific to the facility. It is the responsibility of the principal investigators to ensure compliance and supervise project personnel.

The Dartmouth EH&S office serves as the administrative arm of the IBC and as such oversees the biosafety program at Dartmouth College. Their responsibilities include reviewing and presenting projects to the IBC; advising faculty and staff on proper biosafety procedures for the agents being handled and reviewing and approving the written procedures for all involved researchers and associated personnel. EH&S will also perform periodic inspections of the facility for compliance with the practices outlined in this document and relevant regulations and guidelines.

All personnel conducting ENHANCED BSL2 work recognize the risks inherent in working with human pathogens, and agree to take responsibility for adhering to appropriate protective measures as outlined in institutional guidelines and in this manual.

**E. INFECTIOUS AGENTS**

1. **Modes of Transmission:**

*Please describe the mode of transmission of the biological agents used in this study*

Insert Text Here

1. **Signs and Symptoms of Infection:**

*Please describe the signs and symptoms of clinical human infection.*

Insert Text Here

1. **Methods of Treatment:**

*Please describe the current method of treatment for infected individuals for the agents used in this study.*

Insert Text Here

1. **Methods of Disinfection:**

*Please describe the methods for disinfection of solids and liquids that will be used in this study.*

Insert Text Here

**F. Laboratory Facilities and Safety Equipment.**

**1. Biological Safety Cabinet**: A properly maintained and certified biological safety cabinet is to be used for all manipulations of ENHANCED BSL2 materials.

No ENHANCED BSL2 work is to be conducted on the open bench top. This includes opening containers of potentially infectious materials, pipetting, dilutions, transfer operations, plating, framing, grinding, blending, drying, sonicating, and shaking. ENHANCED BSL2 materials can be centrifuged using centrifuge safety cups or sealed rotor heads with transfer into them using an uncontaminated sealed container.

A labeled spray bottle of 70% ethanol and a container of 10% bleach are kept at each biosafety cabinet for decontamination before and after each work session.

**2. Equipment for Treatment, Disposal and Handling of Wastes**: Appropriate equipment for treatment, disposal and handling of wastes (disinfectants, aspirator flasks, autoclavable waste containers, biohazard red-orange bags, sharp collectors, etc.) shall be readily available inside the ENHANCED BSL2 facility, and are to be used according to procedures described below.

*Please describe the location of the autoclave to be used for these experiments.*

Insert Text Here

**3. Cell Culture Incubators**: ENHANCED BSL2 materials can be kept in cell culture incubators in a closed container. The cell culture incubator is to be cleaned on a scheduled basis.

**4. Water bath.** ENHANCED BSL2 materials can be thawed in the water bath if they are kept in a sealed and water tight container.

**5. Additional Equipment:** Small quantities of ENHANCED BSL2 material in closed containers can be examined or processed in the microscope, refrigerator, freezer or microcentrifuge.

**6. Table-Top Centrifuge**: For centrifugation of ENHANCED BSL2 material, screw capped tubes and aerosol-containing canisters must be used, as well capped centrifuge buckets. If there has been a spill in the hood or concern exists that the outsides of containers to be centrifuged are contaminated, the outsides must also be wiped down with 70% ethanol before removing from the biosafety cabinet. If there has been any possibility of leakage during centrifugation, the inner walls of the centrifuge and the rotor will be immediately decontaminated. Leakages during centrifugation must be reported immediately to the Principal Investigator.

Careful attention must be given to placing a balanced load into the centrifuge. Balance problems will create experimental problems and will increase the possibility of tube breakage. Centrifuge and centrifuge buckets and walls are to be cleaned on a scheduled basis. When a spill of any type occurs, the centrifuge must be cleaned immediately and before any continued use. See appendix for cleaning protocol.

**7. Pipetting Aids:** When ENHANCED BSL2 material is pipetted using disposable pipettes in the biosafety hood, care should be taken to avoid aerosolization. In addition, after pipetting is complete, bleach decontamination of pipettes will be undertaken such as through pipetting the diluted bleach waste solution up and down in the pippetor prior to pipette disposal in a pipette coffin. Care should be taken that liquid is not drawn in the pipette aid. This can be prevented by not over-filling the pipette. If liquid enters the pipette aid it will be collected in the filter enclosed in the aid. You will know this has occurred as the pipette will no longer aspirate or dispense liquid. If this happens, remove the pipette from the unit. Remove the nose cone area of the aid by using a twisting action. Remove the contaminated filter and discard in a biohazard waste container. Use a tissue wetted with alcohol to wipe the inside area before replacing the filter. This is an acrodisc filter with a pore size of 0.45um.

**8. Laboratory Doors**: A large biohazard sign is present on the door to the area in which ENHANCED BSL2 work occurs. The door shall be self-closing and shall have a glass window for sight capability into the room. A red emergency notification card listing the names and phone numbers of the contact persons is also posted.

**9. Sink**: The lab shall contain a sink that is to be used for the disposal of liquid ENHANCED BSL2 waste after proper chemical decontamination, as described below. Bottles of concentrated bleach are stored underneath the sink. Soap and paper towels are available from dispensers near the sink. An eyewash/drench hose is located at a nearby sink located in the laboratory.

**10. Vacuum Lines** are protected with secondary traps and in-line hydrophobic filters.

**11. Facility**: The lab is designed so that it can be easily cleaned and shall be maintained free of trip hazards.

**12. Bench Tops:** No ENHANCED BSL2 work will be conducted on bench tops.

**12. Emergency Eyewash**: An eye wash hoses and safety showers shall be made readily available.

*Please list the locations of the eye wash and safety showers for the research laboratory in question.*

Insert Text Here

**13. Personal Protective Equipment**: When ENHANCED BSL2 work is performed, researchers will wear disposal reusable biosafety gowns and gloves.

**a. Gowns.** All disposable lab gowns will be disposed of with ENHANCED BSL2 waste when contaminated or in need of replacement. Disposable gowns and soiled lab coats will not be worn outside of the lab.

**b. Gloves.** Gloves are disposed of when contaminated, replaced frequently, and removed when work with potentially infectious materials is completed or when leaving the area. Gloves are not worn in the common areas of the lab where ENHANCED BSL2 work is not done. Gloves are not to be used to handle common equipment such as phones, desks, and door knobs. Disposable gloves are not washed or reused. Do not spray gloves with alcohol prior to working. Gloves that fit over the sleeves of the lab gown are recommended.

**c. Face protection.** Face protection (glasses or a faceshield) is used whenever splashes, spatters, or sprays of infectious or other hazardous materials may be generated and contamination to the face (eye, nose, or mouth) can be anticipated.

**14. Labeling**: Equipment used for ENHANCED BSL2 work must be labeled with a universal biohazard symbol and marked ENHANCED BSL2. Labeling should include name, date, and the identity of the material.

**15.** The EH&S Office must be notified of any changes in the procedures or practices contained in this document.

**G. Laboratory Practices**

**1. Immunizations**: It is recommended that those working with human blood, body fluids, tissues, etc. receive the Hepatitis B vaccination. This will be provided free of charge. All laboratory personnel, whether they have direct or indirect contact with Enhanced BSL2 agents, should consult with EH&S and Occupational Medicine regarding possible vaccinations. Information on receiving the vaccine will be provided at the time of BSL2 training. Contact EH&S if you wish to consult with Occupational Medicine.

**2. Laboratory Doors:** Doors are kept closed at all times and locked when laboratory is unattended.

**3.** **Training**: The PI is responsible for ensuring that all lab personnel receive appropriate training before conducting ENHANCED BSL2 work. This includes standard EH&S training plus review of this manual. Personnel are required to read and follow the instructions on practices and procedures in this document.

**4. Pre and Post-Work Cleanup Procedures:** The procedures for pre and post- ENHANCED BSL2 work cleanup include the following:

**Pre-Work Procedures**

1. Clean the work surface with 70% ethanol or 10% bleach.

2. Spray off all equipment and tools required for work with 70% ethanol.

3. Place all equipment and tools required for work inside the hood.

4. If a sharps container is required, place it inside the hood.

5. Waste container(s) is available and lined with a biohazard bag.

6. Check that the biosafety cabinet is functioning properly.

7. Check that vacuum flask is prepared with Wedscodyne and lines are intact.

8. Dress in all required personal protective equipment.

**Post-Work Procedures**

1. Place all contaminated materials in biohazard bags.

2. Clean with 70% ethanol all equipment removed from cabinet.

3. Clean hood with 70% ethanol or 10% bleach.

4. In the event of potential aerosolization, clean glass sash inside and outside with 70% ethanol.

5. Remove personal protective equipment taking care to keep from spreading contamination.

6. Store or discard personal protective equipment. Disposable gowns shall be placed in labeled biohazard bags and stored in designated area.

7. Wash Hands.

**5. Use of Biological Safety Cabinet:** All work with open containers with ENHANCED BSL2 material must be performed in the biological safety cabinet. The biosafety cabinet blower will remain ON when ENHANCED BSL2 work is being done in the cabinet. Decontamination of all biological waste must be performed with pure bleach before removal from biological safety cabinet. All biohazardous materials will be transferred out from the biosafety cabinet only in a leakproof container which has been tightly closed and, if contaminated, decontaminated by spraying all over the outsides with 70% ethanol and/or 10% bleach.

Activities that may disrupt the inward directional airflow through the sash opening of a biosafety cabinet are to be minimized. In particular, repeated insertions and withdrawal of the worker’s arms in and out from the work chamber, improper placement of materials, blockage of the front or rear grilles, or brisk walking past the cabinet while it is in use. Work inside the biosafety cabinet should be performed at least four inches behind the front air intake grille and arms should not rest on the grille. All personnel are responsible for thoroughly cleaning the biosafety cabinet with 70% ethanol and/or 10% bleach after each procedure is completed.

**6. Packaging:** Cultures, tissues, or specimens of body fluids are placed in sturdy containers that prevent leakage during collection, handling, processing, storage, or transport. The shipment and off-campus transport of biohazardous materials is strictly regulated by the US Dept. of Transportation. The EH&S office maintains packaging supplies and expertise for shipping biohazardous materials. Please contact EH&S well in advance of any planned shipments or transport of biohazardous materials off site.

**7. Transport of ENHANCED BSL2 material**: The transport of ENHANCED BSL2 material will be done only in sealed, biohazard labeled containers such as a tupperware box. No transport of unsealed containers which contain ENHANCED BSL2 material are allowed in public spaces. ENHANCED BSL2 materials may not be transported to another lab or research area that is not registered and approved for ENHANCED BSL2 work. Any exceptions require EH&S and/or IBC approval.

**8. Sharps**: No sharps are used when conducting ENHANCED BSL2 work if this is at all avoidable. Instead, use plastic lab ware and avoid scalpels, needles or other such equipment. As a general rule, with any work with human tissue, sharps are avoided and a high degree of precaution must always be taken with any contaminated sharp items, such as broken glass, slides, capillary tubes, and pipettes.

**9. Spills**: All spills must be cleaned up immediately and only by trained properly equipped scientific personnel. Autoclave all toweling and sponges used in the cleanup and wash your hands thoroughly afterwards.

**a. Spills inside the biosafety cabinet**: Immediately stop work and remove hands/arms from BSC after decontaminating any areas that came into contact with spill material. If spill material came into contact with gloves, remove gloves in BSC and leave inside BSC. A 10 minute wait is recommended after leaving BSC in order for any aerosolized material to be filtered by BSC HEPA filters before returning to BSC for spill clean-up. Chemical decontamination shall be performed while the cabinet continues to operate. Remove contaminated gloves and put on a clean pair. Spray and wipe the walls, work surface, and equipment with disinfectant such as 70% ethanol or freshly diluted Clorox with a 10% final concentration. The drain pans and catch basins below the work surface of a Class II cabinet may need to be flooded with disinfectant for a 20 minute contact time before draining.

**b. Large Spills outside the biosafety cabinet:** Report immediately to the principal investigator and the EH&S Office after evacuating the room. If an aerosol risk exists, wait 30 minutes for dissipation before reentering the room to complete the emergency procedures. Medical evaluation and appropriate follow-up will be provided by the Occupational Employee Health Department (650-7018) for any exposure incident. Written records will be maintained.

**c. Equipment Contamination:** Label as contaminated and then wearing protective gear, isolate any leaky container into a plastic bag and decontaminate the spill and all contaminated surfaces following the above procedures.

Equipment shall also be decontaminated before it is sent for repair or for maintenance or moved to another lab area.

**d. Reusable Labware:** Must be completely submerged in a freshly prepared 10% Bleach solution for at least 20 minutes and rinsed with water before taking out of the lab or moving to the BSL2 lab space and being given to the glasswashing staff. This includes tissue culture media bottles, beakers, and any nondisposable pipets. Currently very little reusable labware is used.

**10. Accidents Affecting Laboratory Personnel**: Immediate emergency procedures have to be followed in case of accidents that result in overt exposures of personnel to infectious materials. In case of a splash into an eye, nose or mucous membranes of the mouth, the eyewash located by the sink in 314W will be used immediately. In case of skin exposure, thorough washing with disinfectant soap and water at the ENHANCED BSL2 sink is recommended. In case of parenteral injection (*e.g.*, puncture), forced bleeding is first recommended followed by thorough washing.

Accidents and all spills must be reported immediately to the Principal Investigator and/or lab supervisor. Injured personnel must report for immediate medical evaluation, treatment, and post exposure follow up through the Occupational Employee Health Department (650-7018) between the hours of 7:30am-4:00pm. After hours treatment can be received at the DHMC emergency room (911 or x5555) as appropriate, and written records will be kept. An Accident Report Form must be submitted by the PI to the Office of Risk Management.

**11. Treatment of Contaminated Wastes**: All ENHANCED BSL2 waste in the biosafety cabinet must be identifiable as such and be kept separate from the regular BSL2 and BL1 waste. All ENHANCED BSL2 waste will be removed and treated at the end of each procedure by the scientist. In general, contaminated waste will not be left overnight.

ENHANCED BSL2 wastes and contaminated items will be decontaminated by the scientist before being turned over to custodians or glasswash personnel. All liquid or solid wastes are decontaminated before disposal according to the *Dartmouth College EH&S Hazardous Waste Management, Minimization and Disposal Guide.*

**a. Liquid Wastes**: All liquid wastes generated during ENHANCED BSL2 experiments will be immediately decontaminated inside the biosafety cabinet by mixing with bleach (20% final concentration) for at least 20 minutes contact time. The solution may then be disposed of in the ENHANCED BSL2 sink. The sink must then be decontaminated and washed after the disposal.

Liquids aspirated under vacuum will be collected in a 1 liter aspirator bottle which is placed inside a plastic bucket and initially contains approximately 10 ml of Wescodyne. The aspirator bottle is to be emptied when the volume is not more than 500 ml and replaced by the researchers at least every three months. A secondary bottle and a HEPA filter will be placed between the vacuum source and the primary aspirator bottle.

**b. Non-Sharp Solid Wastes**: All solid wastes contaminated with ENHANCED BSL2 materials must be packed, tagged, transported, recorded, and autoclaved by trained scientists.A leakproof pan will be used to hold the biowaste bags or boxes ready for autoclaving. The logs and charts for each ENHANCED BSL2 waste load will be filed in a designated notebook. Autoclaving will be performed according to validation parameters. Autoclaves will be validated by the scientists for proper temperature and pressure control on a periodic basis. Contact EH&S for validation procedures. If an autoclave is not operating properly, a sign will be posted and the Physical Plant Director will be notified immediately.

Solid ENHANCED BSL2 waste (paper, plastic wrappers, gloves, plastic plates, emptied plastic flasks and falcon tubes) will be collected inside the biosafety cabinet in a small, red bag inside a ENHANCED BSL2 labeled, autoclavable container such as a Nalgene plastic beaker or covered pail. The bag will be twisted closed inside the biosafety cabinet, removed directly into a second red bag, tagged with a numbered, white biowaste label, placed in a leakproof autoclave pan and then removed from the ENHANCED BSL2 facility and autoclaved by the scientist at the completion of the procedure.

All biohazard bags containing ENHANCED BSL2 contaminated materials must be closed before being removed from the ENHANCED BSL2 facility and shall be transported to the autoclave in a secondary leakproof container or a metal autoclave pan; red bags of ENHANCED BSL2 waste will not be carried unprotected in the hallway. It is also preferable to transport materials using a cart. The autoclave log must be filled out for each run and the chart paper saved. See Autoclave log in the Appendix. These records are maintained for 3 years. The scientists are responsible for autoclaving their waste immediately after their experiment.. Once the solid waste is decontaminated, it will be placed inside a regular trash bag and be treated as ordinary trash by the housekeeping staff.

**c. Sharps:** Plastic pipettes and syringes fitted with filters used during ENHANCED BSL2 work will be put into a coffin containing of freshly diluted 10% Clorox solution in the biosafety cabinet. The Clorox solution will be drawn into the entire length of the pipette or syringe. After at least 20 min of soaking, the pipettes and syringes will be carefully drained in the sink. The pipettes will be discarded into a small red bag with the rest of the BSL2 waste and disposed of accordingly.

**12. Aerosols and Pipetting**: Pipetting by mouth is prohibited. Mechanical pipetting devices are to be used. Pipettes contaminated by non-ENHANCED BSL2 materials will be disposed of through the standard laboratory waste. Pipettes contaminated with ENHANCED BSL2 materials shall be washed with a bleach-containing solution after use.

**13. Handwashing and gloves**: ENHANCED BSL2 work requires double-gloving. The outside glove must be removed before leaving the hood and disposed of in the bag for contaminated solid wastes. The inside glove must be removed and disposed of as with all biohazard trash. All personnel must wash their hands after conducting ENHANCED BSL2 work. Gloves are not to be used in handling common equipment such as telephones or incubator doors.

**14. Food, etc.**: Eating, drinking, smoking, handling contact lenses, taking medications, and applying cosmetics are not permitted in the lab area.

**15**. **Housekeeping:** Cleaning and decontamination of work surfaces (benches, BSC, incubator, centrifuge, etc.) will be performed by the scientists, as described in institutional BSL2 policies. When ENHANCED BSL2 work is in progress, custodial and maintenance staff will not be given access to the hood or other areas in which the experiment is being conducted. Otherwise, service and maintenance staff should conduct themselves according to institutional policy.