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**Biosafety Level 2 with Enhances Practices (BSL-2+)**

**Background**

Risk Group 2 agents are those which are associated with human disease that is rarely serious and for which therapies and vaccines exist. Risk group 2 agents may be found in lab research operations that work with infectious microorganisms, as well as those that work with human or animal-derived body fluids, tissues and cells. In general, Biosafety level 2 (BSL-2) is the containment standard of practice for biological materials that may harbor a Risk Group 2 agent. For materials that may harbor a Risk Group 3 agent (i.e., one associated with serious or lethal human disease for which medical interventions may be available; high individual risk but low community risk), BSL-3 containment will be required if the agent is transmitted via inhalation or when required by health authorities. However, there are times when specific Risk Group 2 or 3 agents (or materials that could contain these) may be handled at BSL-2 using enhanced practices in order to further limit the potential for personnel exposure and ensure a greater level of biosecurity. This containment level is often referred to as BSL-2+.

Specific basic research examples warranting BSL-2+ containment include:

* Some use of HIV, HBV or HCV, or patient samples containing these microorganisms.
* Use of any biological agent that requires BS2+ containment in accordance with a federal or state health authority permit (i.e., USDA APHIS, CDC, etc.)
* Use of any emerging infectious agent as determined by the Biosafety Officer &IBC Chair with input from the Principal Investigator

This document outlines the minimum standard of practice to be followed when lab activities have been approved by the GW Institutional Biosafety Committee (IBC) for BSL-2+ containment. Additional requirements may apply based on individual agent/activity circumstances. These will be the outlines in the Principal Investigator’s IBC registration and/or Laboratory Specific Biosafety Manual.

**BSL-2+ Containment Administrative/Operations Oversight**

A designated oversight person must be identified for all research activities being conducted under BSL-2+ containment conditions. In the case of activities taking place in a lab assigned only to a Principal Investigator (PI), this person should be identified by the PI, and should be someone who is knowledgeable and proficient in performing the tasks required for the BSL-2+ activities. If the space is a core or shared lab, the designated oversight person should be the Lab Manager (or equivalent) for the space. (For other scenarios, the Biosafety Officer will assist the PI in identifying a designated oversight person.) The designated oversight person must assure that all personnel working on the BSL-2+ activities adhere to the practices outlined in this document as well as the biosafety manual prepared for the activities requiring BSL-2+ containment, and/or the shared facility that operates at BSL-2+ for all activities.

**Additionally, no materials requiring BSL-2+ containment may be transferred to another lab without prior notification to, and approval by, the IBC**. This notification is necessary in order to assure that permit restrictions are not violated. It is also necessary to assure the receiving lab has the facilities and biosafety support structure in place to work safely with these materials before these materials are in hand.

**Biosafety Manual**

A biosafety manual must be prepared for BSL-2+ activities (or lab facilities that operate at BSL-2+ for all operations). This document should be made available to all BSL-2+ authorized lab personnel both electronically and in hard copy. The manual must include:

* This document
* Pathogen safety data sheets/agent summary statements for all agents requiring BSL-2+ containment
* Technical procedures involving manipulation of infectious agents/samples
* Lab-specific biocontainment procedures including:
* Biosafety equipment acquisition and use (see Attachment A for a template)
* Autoclave operation
* Inventory/activity logging
* Spill and exposure response
* Additional procedures based on risk assessment
* Authorized personnel roster and training/proficiency records

This manual will be reviewed (and that review documented) at least annually by the designated oversight person to assure that information is current and complete. The Office of Laboratory Safety (OLS) can assist with annual review upon request.

**BSL-2+ Lab Personnel**

Laboratory personnel must meet the following criteria to be approved to perform for BSL-2+ activities:

* Be included on an IBC registration with an approved BSL-2+ activity/protocol
* Complete all applicable medical surveillance requirements
* Complete all applicable trainings
* Have a mentor assigned who can evaluate proficiency performing tasks associated with BSL-2+ work
* Review the biosafety manual and sign an acknowledgment regarding the expected standard of practice

Once these actions have been completed, the lab member may enter the BSL-2+ lab for training with their assigned mentor. The training and proficiency testing (qualification) process should include:

1. PI or designated mentor demonstrates procedure to trainee
2. Trainee assists mentor in performing aspects of procedure as appropriate as training progresses
3. Trainee demonstrates proficiency by performing procedure for mentor to observe

In the case of a new protocol to be performed by a lab group not previously approved for BSL-2+, a mentor should be sought who has experience working with agents/activities similar to those planned. Contact the OLS for assistance in identifying prospective mentors.

Once the lab member has demonstrated proficiency in all tasks associated with BSL-2+ work, and this has been documented by the mentor and designated oversight person, the lab member may be cleared for independent access and work in accordance with the lab’s operational policies.

See the “Personnel Onboarding & Qualification” procedure in Attachment B for more information.

In the event that a person has fulfilled the qualifications to work independently, but they have not worked at BSL-2+ for over one year, they must complete a BSL-2+ lab practices reorientation with the designated oversight person. Additionally, the BSO must be consulted to determine if task performance requalification is necessary before resuming independent work.

**BSL-2+ Lab Visitor Management**

For the purposes of this document, a lab visitor is any person other thanBSL-2+ authorized lab personnel. Principal Investigators who do not perform bench work and GW personnel are not considered lab visitors if they will enter or be present in the BSL-2+ area for observation purposes only. Labs that operate at BSL-2+ should be maintained in a restricted access state (i.e. card key, pin lock) at all times. Lab visitors should be kept to a minimum. If lab visitors must be present, this must be cleared with the designated oversight person ahead of time. Visitors must be provided with BSL-2+ visitor awareness training and escorted by a person approved for independent BSL-2+ work.

**BSL-2+ Facilities/Engineering Controls Requirements**

Lab areas where BSL-2+ activities will be conducted are expected to meet the following criteria in order to effectively achieve the intent of enhanced containment practices:

1. The BSL-2+ activity space must have a door. The door must be kept closed and posted while BSL-2+ activities are underway.
2. A sink and properly maintained eyewash need to be available within the BSL-2+activity space. This is necessary to support the intent of keeping materials requiring BSL-2+ practices contained within that space in the event of an exposure.
3. A functional autoclave that can be used for waste treatment needs to be readily accessible (preferably nearby on the same floor) to the BSL-2+ activity space.
4. A biosafety cabinet (BSC) with a current NSF 49 certification must be available in the BSL-2+ space for any manipulations of the materials requiring BSL-2+ containment. When used routinely for BSL-2+ activities, the BSC working surface should be disassembled and cleaned at least annually (preferably just prior to annual recertification).
5. Incubators (if required for BSL-2+ activities) should be available within the BSL-2+ space. If the incubator is “common use”, a dedicated area (or shelf) for materials requiring BSL-2+ containment should be used. Primary containers should be placed (and stored) in appropriate secondary containers when outside the BSC.
6. Other processing equipment needed to support BSL-2+ activities (such as centrifuges) should be located within the BSL-2+ activity space whenever possible. Safety cups or sealed rotors are required for centrifugation of any materials requiring BSL-2+ containment.
7. A properly maintained biological spill kit must be available within the BSL-2+ activity space.

**BSL-2+ Personal Protective Equipment (PPE)Requirements**

To provide primary protection in the event of any spill in the lab, personnel who enter BSL-2+ spaces (whether activities are underway or not) are expected to wear attire that completely covers their legs and feet. Minimum personal protective equipment (PPE) required for handling materials requiring BSL-2+ containment includes the following:

* Wraparound fluid-resistant disposable gown
* Two pairs of fluid-resistant disposable gloves
* Safety glasses required if contacts are worn; strongly recommended for all

Additional PPE requirements may be assigned when determined by risk assessment or by PI preference. These requirements will be included in standard operating procedures section of the Biosafety Manual as necessary.

**BSL-2+ Biocontainment Practice Requirements**

All BSL-2 containment practices should be followed and are not specifically stated here in order to reduce redundancy. However, the practices specifically outlined here are expected to be followed by personnel carrying out BSL-2+ activities in order to provide effective containment of the materials requiring BSL-2+ containment:

1. Glass devices or containers must not be used if non-glass alternatives are available and technically feasible.
2. Sharp devices should not be used unless necessary; consult with OLS before implementing the use of a sharp device.
3. The door must be closed and posted with BSL-2+ signage when BSL-2+ activities are underway. (This signage should be approved by OLS). Only personnel who are approved for BSL-2+ activities should enter the space while such activities are underway.
4. The sink and eyewash must be verified as functional and accessible before BSL-2+work starts.
5. Disinfectants must be EPA-registered for destruction of the agent, or deemed effective for destruction of the agent based on scientific literature (if the agent is not HIV, HBV, or HCV).
6. All solid biohazardous wastes must be collected inside the BSC; serological pipettes must be collected separately inside the BSC.
7. Collected biohazardous wastes must be removed from the BSC at the conclusion of procedures if the space is used for activities other than BSL-2+ activities. If the lab is dedicated for the BSL-2+ activities, wastes may be stored in the BSC only if the collection receptacles are closed and the outside of the collection containers are properly disinfected at the conclusion of procedures.
8. Whenever feasible, solid biohazardous wastes must be biologically inactivated by autoclave prior to submitting these to an outside biohazardous waste contractor for terminal treatment and disposal. (If this is not feasible, waste treatment and handling procedures must be determined in conjunction with OLS before BSL-2+ work is initiated.) The general procedure for autoclave treatment of solid wastes is as follows (to be performed by BSL-2+ personnel only):
9. If wastes have minimal moisture content, 50 ml of water should be added to the bag before closure. Bags should be closed using a rubber band, zip-tie or single knot. Bags should be surface-disinfected and placed in a tray for removal from the BSC and transport to the autoclave.
10. Wastes must be autoclaved such that the bag contents are exposed to saturated steam conditions for at least 15 minutes. This can be verified by running a simulant test pack or “test bag” with the biowaste bags. A “test bag” can be prepared by placing nonhazardous simulant materials (such as paper towels or nonhazardous lab plastics) in an autoclavable biohazard bag and placing a loosely sealed conical within that contains a chemical integrator strip or a biological indicator. These products, as well as simulant test packs, are available through manufacturers like Propper, Steris and 3M. (Contact OLS for assistance in identifying a product if needed.) The “test bag” should be closed in the same manner as the bags containing biohazardous waste.
11. Following autoclave treatment, verify that the load was effectively sterilized based on the results from the “test bag” per the manufacturer’s instructions. If sterility was not achieved, repeat the autoclave treatment cycle. If sterility was achieved, place the waste in the designated pickup container. Log all autoclave runs and results, and maintain these in accordance with lab SOPs. Reminders: Do not autoclave liquid wastes that have been treated with a disinfectant as this may create hazardous conditions. Disinfectant-treated liquid wastes may be discharged to the sanitary sewer via the lab sink unless otherwise specified by Health and Emergency Management Systems (HEMS). If the lab generates other “mixed waste” (i.e., biologically contaminated waste containing a particularly hazardous chemical such a guanidinium thiocyanate), contact HEMS for guidance regarding collection and disposal.
12. When working in the BSC, no items (including gloved hands) maybe removed from the BSC unless these are surface-disinfected first.
13. At the conclusion of procedures, all items in the BSC must be thoroughly surface-disinfected, even if the item will be stored in the BSC. (Storage of items inside the BSC is not recommended, but may be warranted.)
14. Incubators or processing equipment where the materials requiring BSL-2+ containment will be left unattended must be posted with the name of the agent and responsible lab contact if the area is not dedicated to the BSL-2+ activities.
15. Processing equipment must be disinfected at the conclusion of procedures to reduce the potential for cross-contamination of lab materials and personnel exposure to contaminated surfaces.
16. PPE worn for BSL-2+ activities must be removed before exiting the BSL-2+ area. Hands must be washed after PPE removal and before leaving the BSL-2+ area.
17. Stock materials requiring BSL-2+ containment must be stored in a manner that prohibits personnel other than the approved lab users from gaining access to these materials.
18. An inventory listing of materials (i.e., what is in possession and where it’s located) requiring BSL-2+ containment must be generated and maintained. The inventory should be verified at least quarterly when the materials are not actively in use. An activity log to document use and surveillance activities is strongly recommended.
19. In the event that a spill, exposure or “near miss” (unexpected event that could have led to exposure) occurs during the course of BSL-2+ work, lab personnel must report this immediately to the PI and/or designated responsible person for the BSL-2+ area and then proceed to GW hospital (Employee health during normal business hours (202-715-4275, or ER if incident occurs after hours).  Students should consult Student Health Services (Marvin Center, ground floor; 202-994-5300). The Biosafety Officer must be informed as soon as possible in order to determine if reporting requirements apply and to determine whether procedural changes are warranted.

**Related Documents**

* [Risk Assessment from “Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories”](https://labsafety.gwu.edu/sites/g/files/zaxdzs2451/f/downloads/MMR_Biosafety%20RA.pdf)
* [BSL-2 with BSL-3 practices summary based on CDC/NIH “Biosafety in Microbiological and Biomedical Laboratories”, 5th ed.](https://labsafety.gwu.edu/sites/g/files/zaxdzs2451/f/downloads/BSL-2-3_Comparison_0.pdf)
* [Assessing Biological Risks](https://labsafety.gwu.edu/sites/g/files/zaxdzs2451/f/downloads/Assessing%20the%20risks%20%281%29.pdf)
* [Risk Assessment Principles](https://labsafety.gwu.edu/sites/g/files/zaxdzs2451/f/downloads/RA_Principles.pdf)
* [Biosafety Level 2 Manual Template](https://labsafety.gwu.edu/sites/g/files/zaxdzs2451/f/downloads/GWU_Biosafety%20Manual%20Template%20for%20BSL-2%20%281%29.doc)

**Attachment A: Biosafety Equipment Acquisition & Use Summary \_ Example Template**

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| --- | --- |
| **BSL-2+ Operational Profile** | |
| Item | Lab-specific Response |
| Which lab areas are included in the BSL-2+ operations? |  |
| Who is the designated responsible person and what is their contact number? |  |
| Which BSC’s are designated for BSL-2+ work? |  |
| Which incubator (and which area within) is designated for BSL-2+ materials? |  |
| Where is the biosafety signage stored that needs to be posted when BSL-2+ is underway (If not BSL-2+ dedicated area) |  |
| Where is the sink and eye wash located? |  |
| Where is the biological spill kit located? |  |
| Where is the autoclave located for biohazardous waste treatment? |  |
| How will waste will be transported to the autoclave if located outside the immediate lab area? |  |

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| **Personal Protective Equipment (PPE)** | |
| Item | Lab-specific Response |
| What is the product name and ordering number for the fluid-resistant wraparound gown to be used? |  |
| * What use restriction apply (i.e., when must it be replaced, how must it be stored if reused, etc.) |  |
| What is the product name and ordering number for the fluid-resistant disposable gloves? |  |
| What is the product name and ordering number for the safety glasses to be used? |  |
| * What use restriction apply (i.e., how can they be cleaned, stored; when they should be replaced, etc.)? |  |
| What is the product name and ordering number for the face shields to be used? |  |
| * When must these be worn? |  |
| * What use restriction apply (i.e., how can they be cleaned, stored; when they should be replaced, etc.)? |  |
| Is any other PPE required? If yes, what is it, where is it, and when does need to be used? |  |

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| **Disinfection and Biohazardous Waste** | |
| Item | Lab-specific Response |
| What surface disinfectant will be used for BSL-2+ work? What is the contact time? |  |
| * Where is the SDS for the disinfectant? |  |
| * If the product is not ready-to-use, how do you prepare it? What is the shelf-life of the prepared solution? |  |
| If liquid waste will generated, what disinfectant product will be used in the collection vessels and in what ratio? |  |
| Where/how should chemically-treated liquid bio waste be discharged? |  |
| Where are the autoclavable biohazard bags? |  |
| Where is the secondary container for transport of waste? |  |
| Where are used serological pipettes and tips collected in? |  |
| What load verification method is used for autoclave treatment? (Details reading waste bag preparation and autoclave treatment should be fully outlined in the labs; Autoclave treatment SOP.) |  |
| Where are treated solid wastes placed for pickup for terminal treatment and disposal? |  |

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| **Other Lab-Specific Biocontainment Items** | |
| Item | Lab-specific Response |
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**Attachment B: BSL-2+ Personnel Training and Qualification Record**

BSL-2+ Designated Oversight Person: use this document to record all relevant action and qualifying events required for the individual named to be approved for independent BSL-2+ work as defined by the laboratory specific Biosafety Manual. Maintain all complete records with the Biosafety Manual for regulatory review purposes. Please notify the IBC and the Office of Laboratory Safety whenever a person has been added to, or removed from, the roster of BSL-2+ authorized lab personnel.

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| --- | --- |
| Personnel Information | |
| Name: |  |
| Job Title: |  |
| Email and phone |  |
| PI: |  |
| Assigned mentor: |  |
| Agents to be worked and scope of activities to be performed: |  |

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| --- | --- | --- |
| Eligibility Requirements | Date Completed | Verified by (initials) |
| Biological Safety and BBP Training |  |  |
| General Laboratory Safety training |  |  |
| IBC/NIH Guidelines training |  |  |
| Biological Safety Cabinet Training |  |  |
| Lab Specific Biosafety Manual Read |  |  |
| Lab Specific Biosafety Manual Q&A with Designated Oversight Person |  |  |
| Added to the IBC registration |  |  |
| Medical Surveillance Enrollment (if applicable) |  |  |
| Respirator fit testing and training (if applicable) |  |  |

**PROFICIENCY ACKNOWLEDGEMENT**

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ has satisfactorily completed all lab-specific procedural training ( listed and documented on Page 2 of this form). He/she has been observed to be proficient in carrying out all procedures outlined in the Lab Specific Biosafety Manual

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Name of the Designated Oversight Person Signature and Date

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Name of PI Signature and Date

**BSL-2+ AUTHORIZED LAB PERSONNEL CODE OF CONDUCT ACKNOLEDGMENT**

I agree to follow all technical and biosafety procedures as outlined in lab’s specific Biosafety Manual. I understand that my privileges to work on BSL-2+ projects may be revoked if I fail to follow these procedures

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Name of the BSL-2+ Authorized Lab Personnel Signature and Date

**LAB-SPECIFIC HANDS-ON PROCEDURAL TRAINING AND PROFICIENCY DETERMINATION**

The PI and BSL-2+ Designated Oversight Person should list all relevant technical SOPs needed to perform planned activities. When possible, technique trainings should be performed using non-infectious simulant materials. If the individual has prior experience with a procedure/technique, they may be evaluated for proficiency after at least 1 observation is completed.

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| Name of Lab SOP | Observation 1 (Date/Initials) | Observation 2 (Date/Initials) | Observation 3 (Date/Initials) | Observation 4 (Date/Initials) |
| Actions to be taken in event of an emergency |  |  |  |  |
| Knowledge of agent in use and risk exposure considerations |  |  |  |  |
| PPE donning on and off |  |  |  |  |
| Biosafety equipment acquisition and Use |  |  |  |  |
| Autoclave treatment of biohazardous waste |  |  |  |  |
| Inventory/Activity logging |  |  |  |  |
| Spill & exposure response |  |  |  |  |
| Centrifuge use |  |  |  |  |
| BSC use |  |  |  |  |
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