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| File:Biohazard symbol (red).svg - Wikipedia Contact InformatioN  |  |  | | --- | --- | | During Normal Hours | | | Name | Enter Text Here | | Position | Enter Text Here | | Phone | Enter Text Here | | Email | Enter Text Here | |  |  | | After Hours | | | Name | Enter Text Here | | Position | Enter Text Here | | Phone | Enter Text Here | | Email | Enter Text Here |  SOP Review Dates: Lab: Enter Reviewer Name & Review Date Here  EHS & EM: EHS & EM Signature Here. | |  | | --- | | Enter Lab Name HereBiosafety level 2Standard Operating Procedure |  Scope: This Standard Operating Procedure (when signed by EHS&EM) serves as this lab’s authorization to perform work with the herein named Risk Group 2 and 1 agents using Biosafety Level 2 practices and serves as the lab-specific biosafety manual. This document is required for review by each member of the lab working with these materials prior to beginning work and as a supplement to the lab member’s hands-on safety training. Each lab member should review this SOP once annually and sign the final page once complete. Brief Description of Work: Please enter a brief description of your lab’s work and which agents you will be using. |

### Risk Assessment

A risk assessment should be conducted to identify the hazards associated with all biological agents utilized in the laboratory. This assessment should examine the nature of the agent itself and any procedures that could potentially lead to exposure. The cause of most laboratory acquired infections are often difficult to determine. Unlike apparent injuries (needlestick injuries, animal bites, etc.), inhalation of infectious aerosols may go unnoticed at the time of exposure. Labs should use these assessments to alert their staff to the hazards associated with specific agents and/or procedures and to integrate mitigation measures (e.g., use of safety cups while centrifuging infectious material) into their protocols.

**Assessment Considerations**

**Agent Awareness**

* What is the risk group of the agent?
* Is there an agent summary available from the CDC or vendor?
* What is the natural route of transmission for the agent?
* What are the symptoms of an exposure to the agent?
* Are vaccinations or treatments available for the agent?
* Is the agent hazardous to a particular group of people (e.g., immunocompromised)?
* Has the organism been modified in any way?
* Are there any transgenes expressed; do they increase risk (e.g., oncogenes)?

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**Procedure Awareness**

* Are aerosols generated from centrifugation, sonication, or vortexing?
* What is the working volume and concentration of the agent?
* Will animals be a part of the work?
* Will sharps be a part of the work?
* Is appropriate PPE available?
* Are appropriate disinfectant measures in place?
* Have all waste streams been properly addressed?
* Should the procedure be conducted within a biosafety cabinet?

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**Routes of Exposure**

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|  | It is estimated that up to 65% of laboratory acquired infections are related to the inhalation of bioaerosols. Consider measures to limit aerosol production and contain aerosol release. |
|  | Needlesticks are another significant source of exposures. If needle use cannot be eliminated, consider the use of safety-engineered sharps, and emphasize proper sharps disposal. |
|  | Inappropriate use of PPE can lead to unintentional ingestion of biological materials. Contaminated PPE must be removed prior to leaving the laboratory or touching common surfaces (e.g., door handles, equipment controls). |

### Administrative Controls

**Training, Medical Surveillance, and Vaccinations**

* All lab personnel working with BSL-2 listed materials must be appropriately trained. This includes OSHA and University required training, review of this SOP, and hands-on laboratory-specific training provided by senior lab staff. The PI and/or the laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 material.
* Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents present in the laboratory. Contact [labsafety@appstate.edu](mailto:labsafety@appstate.edu) for questions.
* Personnel who work with human materials, including blood, body fluids, tissue, primary cells, established cell lines or other potentially infectious material should be offered the Hepatitis B Vaccine and enrolled in the university Bloodborne Pathogens Program.
* As personal health status may impact an individual’s susceptibility to infection or prevention and treatment options, all laboratory personnel, particularly immunocompromised and women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Such individuals are encouraged to voluntarily self-identify to Employee Health (FastMed) or Student Health for consultation.

**Signage**

* The entrance to the laboratory must be identified with the appropriate biohazard signage, which lists the biohazardous materials in use and the names and phone number(s) of personnel to contact in case of an emergency, and any special entry requirements. A template is provided at the end of this document.
* All equipment including, but not limited to, BSC, centrifuges, incubators, refrigerators, freezers, and liquid nitrogen tanks used in the manipulation or storage of infectious materials must be appropriately labeled with a biohazard label containing the international biohazard symbol, or the word “Biohazard”, lettering and symbols in a contrasting color.

**Procedural Requirements**

* Occupants must not eat, drink, apply makeup/lip balm, or handle contact lenses in the lab.
* Food and drink for human consumption must not be stored in the lab. All refrigerators/freezers containing biological material must be marked with the biohazard symbol and possess signage indicating that storage of food or drink is prohibited.
* Lab members must wash their hands after stopping work, at the time of a glove change, and before leaving the lab. If a sink with running water is not immediately available in a work area, a waterless hand sanitizer must be provided. If hand sanitizer is utilized, lab personnel must wash their hands with soap and water at the nearest sink upon exiting.
* Mouth pipetting is not permissible; mechanical devices must be used.
* An appropriate disinfectant is utilized to clean work surfaces following BSL-2 manipulations. If 10% bleach solutions are used, the dilutions must be prepared fresh weekly (at minimum) and labeled with the date they are prepared. If a disinfectant other bleach is utilized, it must be an EPA-registered disinfectant (e.g., Vindicator) and applied following manufacturer’s specifications.
* Due to concerns over their effectiveness and potential health risks, EHS & EM strongly discourages the use of UV lights as a method of decontamination.
* All procedures are performed in a manner that minimizes the creation of aerosols. Aerosol generating procedures (e.g., blending, sonicating, vortexing, etc.) must be performed in a BSC or other containment device.
* Additional personal protective equipment, which may include a shield or goggles and mucous membrane protection, e.g., a molded face mask or equivalent, is utilized for procedures that do not fit into a BSC or other containment device or where containment equipment is unavailable.
* Biological materials transported between laboratories or on-campus facilities should be placed in leakproof secondary containment labeled with the universal biohazard sticker. The secondary containment must be decontaminated prior to exiting the laboratory.

**Sharps**

* Plasticware should be substituted for glass wherever possible (e.g., blood tubes, capillary tubes, Pasteur pipets, pipets, centrifuge tubes, etc.), especially when using with human tissue culture or infectious materials.
* When eliminating a sharp is not feasible, a safety-engineered sharp should be substituted (e.g., disposable/retractable scalpel instead of a razor blade, etc.).
* Needles must not be bent, sheared, or recapped. They should be disposed of in a readily accessible, puncture resistant container marked with a biohazard symbol immediately following use.
* Sharps containers must never be overfilled; they should be stored in the upright position and closed and sealed when the contents near the “full” line on the container.
* Contaminated glassware must be disinfected prior to discarding into a broken glass container.
* A mechanical means (forceps, dustpan and brush, cardboard “shovel”) must be used to clear broken contaminated glassware. Never handle broken glass with the hands, even if wearing gloves.

### Engineering Controls

* The laboratory is designed so that it can be easily cleaned.
  + Spaces between benches, cabinets, and equipment are accessible for cleaning.
  + Floors should be impervious to harsh chemicals, designed to prevent moisture or debris build-up, easily disinfected. Carpets and rugs are not appropriate for laboratory use.
  + Benchtops are impervious to water, resistant to heat, organic solvents, acids, alkalis, and other chemicals and easily cleaned and disinfected.
  + Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
* Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
* Handwashing facilities and emergency irrigation equipment are available in the laboratory.

A picture containing text, first-aid kit, clipart

Description automatically generatedA sink for handwashing is available in the laboratory. Lab members must wash their hands after stopping work, at the time of a glove change, and before leaving the lab. If a sink with running water is not immediately available in a work area, a waterless hand sanitizer must be provided. If hand sanitizer is utilized, lab personnel must wash their hands with soap and water at the nearest sink upon exiting.

A picture containing text, clipart

Description automatically generatedAn eye wash station or an eyewash/drench hose unit must be available in areas wherever there is a possibility of exposure to biological material. The eyewash or drench hose must be capable of providing a 15-minute flush of tepid water to the eyes and/or body and free of any obstructions. A weekly documented operational check of all eyewash units is required.

Icon

Description automatically generatedAn emergency shower should be available. The emergency shower should be able to provide a 15-minute flush of tepid water, located within 10 seconds (about 55 feet) of the lab, free of any obstructions, and flushed weekly to ensure proper working conditions.

* Biosafety Cabinet(s) (BSCs) are used to help mitigate the risks associated with the inhalation of aerosols containing potentially infectious agents and to protect the research materials from contamination.
  + Our BSC(s) are: Class 1  Class 2, Type: A1 A2 B1 B2
  + BSC(s) must be certified annually. Our BSC(s) were last certified on: Click or tap to enter a date.
  + Keep in mind that BSC(s) offer protection to the worker (and class 2 cabinets offer protection to the product) only when used correctly:
    - Work 6 inches or more into the depth of the cabinet, not right at the edge.
    - Keep the sash of the cabinet lowered as far as possible. This helps maintain the intended airflow and helps protect your face from any splashes.
    - Do not block the front grill or rear vents with your arms or other materials.
    - Keep bulky objects out of the cabinet. Only transfer in objects you need.
    - Regularly disinfect the surfaces of the cabinet with an appropriate cleaner.
    - Create a flow to your work going from your most clean materials to your most dirty.
  + Chemical use in BSC cabinet should be limited. A fume hood should be utilized whenever possible.
* When centrifuges are used for processing potentially infectious materials outside a BSC
  + Sealed rotors or safety cup must be utilized.
  + Tubes, bottles, conicals, and rotors is inspected for cracks and deformities before each use.
  + Cups/rotors are loaded/unloaded inside the BSC in case of leakage.
  + Lab staff should wait 10 minutes after a run to allow aerosols time to settle before unloading.
  + In the event of an accidental release (e.g., spill in centrifuge, broken tube, rotor failure), users should wait at least 30 minutes for aerosols to settle before decontamination.
* All vacuum lines are protected from contamination with liquid disinfectant traps and in-line HEPA filters. Replace HEPA filters every six months to a year, depending on the number of users, cleanliness of the unit and laboratory and incubator design.
* Directional airflow (into the lab) is highly recommended for BSL-2 labs to minimize the potential release of aerosols into public spaces.

### Personal Protective Equipment

* A picture containing text, screenshot, electronics

  Description automatically generatedA fastened lab coat with sleeves extending to the wrist is required to work in a BSL2 lab.
  + Lab coats are provided and laundered by Cintas.
  + Lab coats should be regularly dropped off at your building’s drop point for laundering. Our drop-off location is Enter Location Here
  + Lab coats should never be taken home.
  + Long pants and close-toed shoes are required. Long hair should be pulled back or restrained.
* Use proper ‘donning and doffing’ technique to put on and take off PPE. This includes turning PPE inside out as it is taken off to avoid skin contact with any contaminated surfaces.
* Safety glasses are required. Safety goggles or a faceshield must be worn when working with biological material outside of a biosafety cabinet as exposure to splashes, sprays, or aerosols are more likely.
* Glovesare also required personal protective equipment (PPE) in a BSL2 lab.
  + - Care should be taken to choose an appropriate glove for materials being used.
    - Nitrile gloves are suitable for biological materials alone, but keep in mind the compatibility of the glove type with any chemicals being used in addition to biological specimens.
    - Consult the glove manufacturer and the chemical SDS to determine [glove compatibility](https://ehs.unc.edu/wp-content/uploads/sites/229/2015/09/Ansell_8thEditionChemicalResistanceGuide.pdf).
    - Never save and/or re-use disposable gloves. Dispose of contaminated gloves as biological waste.
    - Lab members must dispose of a pair of gloves and don a new pair when a glove is noticeably contaminated or when the glove’s integrity is compromised.

### Laboratory Access

* Access to the laboratory is subject to PI/laboratory supervisor approval and is limited to trained or escorted personnel.
* Lab doors should be self-closing and locked when no lab member is present.
* Lab doors must be closed when work with BSL-2 material is in progress.

### Biological Waste

|  |  |  |  |
| --- | --- | --- | --- |
| **Type of Waste** | **Autoclave\*** | **Chemical Disinfection\*\*** | **Vendor**  **Pick-up\*\*\*** |
| **Liquid** |  |  |  |
| **Solid** |  |  |  |
| **Contaminated Equipment/PPE** |  |  |  |
| **Sharps/Broken Glassware** |  |  |  |

* All potentially biohazardous waste must be disposed of in accordance with university, local, state, and federal regulations.
* For drain disposal, the waste must be free of any prohibited material and pre-treated as specified in the [Drain Disposal Guidance](https://appsafety.appstate.edu/sites/default/files/flyer_-_drain_disposal_guidance_-_ehsem.pdf).
* Where waste must be transported for treatment, it must be appropriately packaged and labeled.

\*This lab uses an autoclave to treat biological waste:

1. Autoclave location: Enter location
2. Autoclave Procedures: Enter procedure details
3. Autoclave Safety:
   1. Ensure proper autoclave training has been completed before use.
   2. Refer to the [Autoclave Safety Document](https://appsafety.appstate.edu/sites/default/files/flyer_-_autoclave_safety_ehsem.pdf) for safety considerations and monitoring requirements.

\*\*This lab uses chemical disinfection to treat biological waste:

1. Disinfectant(s) utilized: Enter disinfectant(s) used.
2. Disinfection procedures: Enter disinfection procedure
3. Note that bleach (sodium hypochlorite) solutions become less effective over time. A fresh bleach solution should be prepared and dated at least weekly.

\*\*\*This lab contracts with a vendor to pick up biological waste:

1. Vendor Name: Enter Vendor Name
2. Pick-up Schedule: Enter schedule details

**Liquid Waste**

* + Containers must be labeled with the biohazard symbol and placed in secondary containment (if the container is glass and stored on the floor or near any drains).
  + Liquid waste should never be discarded into biohazard bags.

**Solid Waste**

* + Contaminated solid waste is any waste that has been in contact with biohazardous or potentially biohazardous material including, but not limited to, vials, centrifuge tubes, slides, gloves, paper towels, etc.
  + Such materials should be discarded into biohazard bags.
    - Contaminated pipette tips and serological pipettes should be collected in a pipet keeper box or a plastic bottle (with a biohazard label) and then placed in the biohazard waste to prevent puncturing the biohazard liner bag.
    - Pipette tips or pipettes used for water and other non-hazardous materials can be discarded into broken glass bins.

**Sharps**

* Because of the hazard posed by contaminated sharps (e.g., broken glass, Pasteur pipettes, needles), these materials must be placed in a puncture-resistant, leak-proof container.
* The puncture-resistant container must be marked with a biohazard symbol.
* The container should be readily accessible to the user, kept in the upright position, and replaced when full (as indicated by the container labeling).
* Contaminated glass such as Pasteur pipettes and slides may be discarded into a broken glass bin if appropriately disinfected beforehand.

### Spill Procedures

**Spills outside of a biosafety cabinet**

* 1. Remove any contaminated clothing and PPE and discard as biohazardous waste.
  2. Evacuate the space for 30 minutes to allow aerosols to settle.
  3. Enter the lab space wearing appropriate PPE
  4. Cover the spill with paper towels or other absorbent material to absorb the spill and prevent further aerosolization.
  5. Pour 10% bleach on the spill starting out the outer edges and working inwards. For large spills undiluted bleach should be used. If a disinfectant other than bleach is utilized, it must be EPA-registered and applied following manufacturer’s specifications.
  6. Allow paper towels soaked with disinfectant to stand for 20 minutes.
  7. Clean up the bleach and spill using absorbent material.
  8. Discard all spill clean-up materials as biohazardous waste
  9. Use tongs or a brush and pan to remove any sharps and discard into a sharps container.
  10. Wash the affected surface with soap and water three times.

**Spills inside of a biosafety cabinet**

* 1. Wait at least five minutes to allow the BSC to contain aerosols before cleaning.
  2. Wear laboratory coat, safety glasses and gloves during cleanup.
  3. Allow BSC to run during cleanup.
  4. Cover the spill with paper towels or other absorbent material to absorb the spill and prevent further aerosolization.
  5. Apply 10% bleach on the spill starting out the outer edges and working inwards. If a disinfectant other than bleach is utilized, it must be EPA-registered and applied following manufacturer’s specifications.
  6. Allow paper towels soaked with disinfectant to stand for 20 minutes. If bleach or another corrosive disinfectant is used, wipe spill area and disinfected equipment with 70% ethanol or water to prevent damage to the BSC.
  7. Wipe up spillage with disposable disinfectant-soaked paper towels and discard as biohazardous waste. Do not place your head in the cabinet to clean the spill; keep your face behind the viewscreen.
  8. Wipe the walls, work surfaces, and any equipment in the cabinet with disinfectant-soaked paper towels.
  9. Discard contaminated disposable materials using appropriate biohazardous waste disposal procedures.
  10. Remove any sharps using tongs or forceps and discard directly into a sharps container.
  11. Place contaminated reusable items in biohazard bags or autoclavable pans with lids before autoclaving.
  12. Expose non-autoclavable materials to disinfectant (appropriate contact time) before removal from the BSC.
  13. Remove protective clothing and segregate for disposal or cleaning.
  14. Run BSC 10 minutes after cleanup before resuming work.

**Spill or breakage during centrifugation**

1. Wait at least 30 minutes for aerosols to settle before decontamination.
2. Don the appropriate PPE.
3. Remove any sharps using tongs or forceps, disposing in a sharps container.
4. Disinfect the centrifuge and buckets with 10% bleach or an EPA-registered disinfectant, such as Vndicator or Cavicide, followed by 70% ethanol to prevent damage to the centrifuge.
5. If possible, the centrifuge buckets/rotors should be transferred to a BSC to complete the disinfection process.

### Exposures

* In case of a life-threatening emergency, call 911 or 828-262-8000.
* Report all injuries or illness to your supervisor immediately.
* Students may seek appropriate medical attention from Student Health Services during clinic hours (**Mon-Fri:** 8:00 am – 4:00 pm; **Sat-Sun**: (Limited Services)) 8:00 am – 11:00 am.
* Seek appropriate medical attention from the following authorized primary care physicians:
  + **Mon-Fri**: 8:00 am - 8:00 pm; **Sat-Sun**: 8:00 am - 4:00 pm

[Fast-Med Urgent Care](https://www.fastmed.com/urgent-care-centers/boone-nc-walk-in-clinic/)

178 Hwy 105 Ext, Suite 101

Boone, NC 28607

828-265-7146

* + **Non-Clinic Hours**

[Watauga Medical Center Emergency Department](https://apprhs.org/wataugamedical/)

336 Deerfield Road

Boone, NC 28607

828-262-4100

* Complete the required documentation ([Initial Notification of Incident Form](https://hr.appstate.edu/hr-services/leave-management/workers-compensation/initial-notification-incident-form-0)), Supervisor's Accident/Illness Investigation Form, Medical Records Release Form, and Witness Statement) and forward to the Office of Human Resources - Workers' Compensation (Carolyn Bosley (262-6488)) within 24 hours of the incident or as soon as possible.
* Notify the Institutional Biosafety Council (IBC) if the exposure involved any recombinant nucleic acid material.
* Return to work after your medical treatment unless your authorized physician provides you with a written authorization prohibiting your return to work. Provide a doctor's note, to your supervisor, returning you to work **with no restrictions or stating any medical restrictions** placed upon you as a result of the work-related injury.
* Keep supervisor notified of any changes in medical condition or any concerns in reference in your case.

### Door Sign

* A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present (see below). Posted information includes: the laboratory’s Biosafety Level, the supervisor’s or other responsible personnel’s name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy. A template is provided at the end of this document.

### References

1. Biosafety in Microbiological and Biomedical Laboratories, 6th Edition. Centers for Disease Control and Prevention and National Institutes of Health. PDF Available here: <https://www.cdc.gov/labs/BMBL.html>
2. Prudent Practices in the Laboratory; Handling and Disposal of Chemicals. National Research Council. PDF Available here: <https://www.nap.edu/catalog/4911/prudent-practices-in-the-laboratory-handling-and-disposal-of-chemicals>

### Signature Page

The following personnel attest that they have read, understand, and agree to adhere to the biosafety policies and procedures contained within this BSL-2 SOP.

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| --- | --- | --- | --- |
|  | Name (Print) | Signature | Date |
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### Enter Lab Name

**BIOHAZARD**

****

Biosafety Level 2

Authorized Personnel Only

|  |  |  |  |
| --- | --- | --- | --- |
| Contact Information | |  | Agents Present |
|  | |  |  |
| *During Normal Hours* | |  | List Biological Agents Here. |
| Name | Enter Text Here |  |
| Position | Enter Text Here |  |
| Phone | Enter Text Here |  |
| Email | Enter Text Here |  | Requirements Prior to Entry |
| *After Hours* | |  |
| Name | Enter Text Here |  |  |
| No food or drink allowed.  List required PPE for entry here. |
| Position | Enter Text Here |  |
| Phone | Enter Text Here |  |
| Email | Enter Text Here |  |
|  | | | |