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###### University of Utah

**Biosafety Manual and Standard Operating Procedures**

**Biosafety Level 2 Laboratory**

of

*Dr. (name of PI)*

(title)

(Institute or department and building, room location)

*(date developed)*

*(annual review/revision date)*

This document serves as a template for a Biosafety Manual and Standard Operating Procedures for BSL-2 laboratories at the University of Utah. It reflects the requirements of the University of Utah Institutional Biosafety Committee (IBC) for appropriate BSL-2 Biosafety manuals and SOPs and OSHA for a laboratory exposure control plan. PIs should customize this document for their laboratory requirements and incorporate it into their Institutional Biosafety Committee (IBC) registration. November 2024 revision.

**\*\*\*Please edit or delete all highlighted sections and remove highlighting**

# Principal Investigator’s Certification

I hereby certify that I have reviewed the contents of this manual and that it reflects my current operating practices. Review must be completed at least annually.

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| --- | --- |
| **Date Reviewed:** | **PI Signature:** |
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# Biological Agents Used in this Laboratory

Note: Registration of materials and addition or deletion of personnel and lab rooms must be processed through SciShield (formally BioRAFT) or ERICA: <https://ibc.utah.edu/ibc-registration.php>

|  |
| --- |
| **Biological Material**  ALL material listed for use in this lab **suite:** e.g., Microorganisms, Recombinant Nucleic Acids (e.g. viral vectors), Acute Biological Toxins, Human-derived materials, Non-human primate blood, tissues, or body fluids |
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**Signature and Acknowledgement of Risk**

We, the undersigned, understand that the agents used in this laboratory and described in this manual are potentially hazardous.  We have read and understand this manual and agree to follow the stated policies and procedures.  All laboratory personnel are required to review this manual annually and complete training requirements (e.g. BBP, BSL-2, ABSL-2) determined by the risk assessment: <https://ibc.utah.edu/training.php>.

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| --- | --- | --- | --- |
| **Name** | **Signature** | **Date Reviewed Manual** | **Date completed BBP/BSL-2/ABSL-2 training (as applicable)** |
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# Emergency Information

# Emergency Phone Numbers

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| --- | --- |
| Fire and Medical Emergencies | 911 |
| Police | 911 |
| *Laboratory PI Home Phone*  *Office Phone*  *Cell Phone* |  |
| *Laboratory Manager Home Phone*  *Office Phone*  *Cell Phone* |  |
| Occupational Medicine | 801-213-9777 |
| Campus Police Department | 801-585-2677 |
| Environmental Health and Safety (Main Number) | 801-581-6590 |
| Biosafety Officer (EHS) | 801-585-9325 |
| Associate Biosafety Officer (EHS) | 801-585-3345 |

Emergency Equipment:

**telephoneS** are located ***(insert location).***

**fire extinguisher** is located (***insert location***)**.**

**eyewash** is located **(*insert location)*.**

**SAFETY showeR** is located ***(insert location)***

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***Emergency Assembly Point for Laboratory Personnel*:**

**University of Utah**

**Biosafety Level 2 Standard Operating Procedures**

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1. **Requirements and Purpose**

The standard operating procedures (SOPs) described in this manual apply to all research staff, hosted visitors and guests, volunteers, building staff, and service staff who enter the laboratory. Review of this Manual is mandatory for all employees and staff working in the BSL-2 laboratory of Dr. (*name of PI*). The Manual has been customized to provide lab-specific provisions to identify and protect all personnel who may be at risk of exposure. This Manual must be updated at least annually, and whenever there are changes in laboratory procedures that may change a worker’s exposure. A copy of this Manual must remain in the lab, and must be accessible to lab personnel.

Research involving recombinant/synthetic nucleic acids, infectious agents, creation of transgenic or knockout animals, acute biological toxins, unfixed non-human primate materials, or human blood, cells, or unfixed tissues must submit a research registration through SciShield (formally BioRaft) to the Biosafety Office. This work may require prior review and approval by the Institutional Biosafety Committee, according to the [*NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*](https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf) and must be renewed at a regular schedule determined by the committee (typically every 3 years). Changes to SOPs and protocols that include new agents or risks must be submitted as a registration amendment for review by the IBC.

This document is a lab-specific manual, which supplements the [University of Utah Biosafety Manual](https://ibc.utah.edu/biosafety-policies.php) to meet the guidelines of the [*Biosafety in Microbiological and Biomedical Laboratories*, 6th ed](https://www.cdc.gov/labs/bmbl/index.html) and the guidelines and requirements of [OSHA’s Laboratory Safety Guidance](https://www.osha.gov/sites/default/files/publications/OSHA3404laboratory-safety-guidance.pdf).

# Training and Hepatitis B Vaccination Program

All personnel who work in the laboratory must receive adequate instruction from their supervisor prior to beginning work. Some training is required annually. Each lab will require different trainings (edit as applicable). The minimum requirements for qualification to work in the (*name of PI*) BSL-2 lab are:

1. Initial and Annual EHS Bloodborne Pathogen (BBP) training\*, if working with human-source material (including cell lines) or other potentially infectious material (OPIM).
2. Initial EHS Biosafety Level 2 Training: repeated every 3 years. This is available as a combined training with BBP.
3. Initial EHS Animal Biosafety Level 2 Training: repeated every 3 years
4. Initial and Annual Laboratory Specific Training (Risks associated with the hazards/agents used in the lab, SOPs, Spill and Exposure Procedures) including annual review of this manual. Note: Appendix 1 contains information about conducting a risk assessment.
5. Shipping Training: Training is required to commercially transport infectious materials, as well as shipping anything on dry ice. Training is available from EHS via Bridge and must be repeated every 2 years. Note: Category A infectious substances must be packaged and shipped by EHS.
6. (*List as many as you need)*

No employee will be trained to work in the lab without the express permission of *Dr. (name of PI)*. *Dr. (name of PI)* will provide information and arrange for training at the time of an individual's initial assignment to the lab. *Dr. (name of PI)* will arrange for refresher training at least annually and when there are any changes in processes or procedures. Documentation of training must be uploaded to the laboratory registration in [SAM](https://sam.ehs.utah.edu/ehsa/). New SOPs and protocols must be approved by the PI before initiation.

\* BBP Training will include information about reducing risk by obtaining a vaccination for Hepatitis B. All employees who have potential exposure to blood or other potentially infectious materials, including human and non-human primate cell lines, will be offered the Hepatitis B vaccine by the RedMed Clinic, at no cost to the employee. The vaccine will be offered within 10 working days of their initial assignment to work involving the potential for occupational exposure to blood or other potentially infectious materials following completion of the EHS bloodborne pathogens training.

Vaccination is encouraged unless: 1) documentation exists that the employee has previously received the series, 2) antibody testing reveals that the employee is immune, or 3) medical evaluation shows that vaccination is contraindicated.

After completing the BBP training provided by EHS, the employee will be contacted by Occupational Medicine to enroll in their Hepatitis B vaccination program, where they will be able to request vaccination or decline the vaccination. Employees who decline may request and obtain the vaccination at a later date at no cost. Documentation of refusal of the vaccination will be sent to the employee by email and kept in the Occupational Medicine Open Range system. Copies of vaccination records and/or declination statements can be obtained by sending an email to [occupational.health@hsc.utah.edu](mailto:occupational.health@hsc.utah.edu).

# Agents Used in the Lab

This Manual applies to work with *(name of agent(s)): Complete 1-4 below for EACH biological hazardous agent used in the lab.*

1. Risk Assessment

*Insert Description Here: include consideration of parent and recombinant agent pathogenicity, virulence, infectious dose, route of transmission and exposure sources, genetic alterations that alter the pathogenicity, host range, availability of vaccines and prophylactic measures, and stability. See Appendix 1 for guidance on completing a risk assessment.*

*Consider if the agent is more hazardous to immunocompromised individuals. If so, include a statement like that below:*

Individuals who are immunocompromised (e.g., individuals with immunologic conditions or are being treated with immunosuppressive agents, pregnant woman, individuals with AIDS) should be advised of the risk of working with this agent, and it is recommended that they consult with a healthcare provider.

1. Signs and Symptoms of Infection

*Insert Description Here*

1. Prophylaxis and Treatment Options

*Insert Description Here*

1. Methods of Disinfection and Inactivation

*Insert Description Here*

For disinfecting blood or other potentially infectious materials covered by the OSHA bloodborne pathogen standard, an EPA-approved must be used: <https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants>

Check expiration dates on commercial disinfectants: stock bottles of bleach should be discarded no later than one year after the date of manufacture or 6 months after purchase, whichever comes first. Note: Dilutions of bleach should be made fresh at least twice per week.

1. **Exposure Control Plan** (include this section if the lab works with human blood and other potentially infectious materials (OPIM), including human and non-human primate cell lines). Remove this section if you do not work with human source material or OPIM.

Laboratories working with human blood and other potentially infectious materials (OPIM), including human and non-human primate cell lines, must comply with OSHA's Occupational Exposures to Bloodborne Pathogens in Title 29 Code of Federal Regulations 1910.1030 and as revised in 2001 by the Needlestick Safety and Prevention Act P.L. 106-430. One requirement is to develop and implement an Exposure Control Plan (ECP), which is reviewed annually. This Biosafety manual has been adapted to serve as an ECP, and annual review is required. It includes Appendices 9 and 10, the “Sharps Injury Log” and “Annual Review of Safe Sharp Devices.”

This biosafety manual describes work practices, engineering controls, and PPE designed to reduce or eliminate employee exposure to bloodborne pathogens. All human blood and OPIM are considered to be infectious for Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV), and other bloodborne pathogens, and will be treated as if infectious, i.e. with universal precautions. See the [University of Utah’s Exposure Control Plan](https://ibc.utah.edu/biosafety-policies.php) as a reference.

**Roles and Responsibilities**

* 1. (*Name of Individual*) is responsible for the implementation of this ECP.
  2. *(Name of Individual*) will maintain, review, and update this ECP at least annually, and whenever necessary to include new or modified tasks and procedures.
  3. Those employees who have occupational exposure to blood or OPIM must comply with the procedures and work practices outlined in this ECP.
  4. (*Name of Individual*) will maintain and provide all necessary personal protective equipment (PPE), engineering controls (e.g., sharps containers), labels, and red bags as required by the standard. This will include ensuring that adequate supplies of the aforementioned equipment are available in the appropriate sizes.
  5. (*Name of Individual*) will be responsible for documentation of annual consideration and implementation of appropriate engineering controls, including safer sharp alternatives (Appendix 10).
  6. (*Name of Individual*) will be responsible for solicitating input from employees regarding identification, evaluation, and selection of effective engineering and work practice controls.
  7. (*Name of Individual*) will be responsible for ensuring that all medical actions required are performed.
  8. (*Name of Individual*) will be responsible for arranging training, documentation of training, and making the written ECP available to employees, as well as OSHA representatives, if requested.

1. **General BSL-2 Laboratory Practices, Including Engineering and Work Practice Controls**

# Work with the agent will be performed in *(insert building and room number)*. Practices must adhere to the standards described in the University of Utah Biosafety Manual (<https://ibc.utah.edu/biosafety-policies.php>). These include:

1. It is the responsibility of each lab member to carefully consider every action taken in the BSL-2 lab and its potential impact on possible exposure or contamination, and to follow established Standard Operating Procedures (SOPs) and protocols diligently and without variance.
2. Access to the laboratory is restricted to authorized personnel only.
3. No eating, drinking, chewing gum, smoking, handling contact lenses, or applying cosmetics in the lab at any time. Food should not be brought into the lab for storage or later use. Food is stored outside in areas designated specifically for that purpose.
4. A biosafety cabinet is used for procedures that result in creating infectious aerosols or splashes or when high concentrations or large volumes of infectious agents are used. This includes pipetting, opening containers of infectious materials, inoculating animals, mixing, sonicating, and harvesting infectious tissue.
5. Personnel must wear PPE appropriate for their work, as determined by the lab’s PPE assessment in [SAM](https://sam.ehs.utah.edu/ehsa/), and it must be provided in the appropriate sizes at no-charge by the Principal Investigator. It must be donned upon entering the BSL-2 laboratory.

* At a minimum, they must wear a lab coat, gloves and safety glasses, in conjunction with long pants and solid, closed shoes.
* Describe any additional lab-specific or procedure-specific PPE here.
* Lab coats with cuffed sleeves or disposable sleeve covers with tight cuffs are recommended.
* No skirts, shorts, or sandals are to be worn in lab. No bare legs, ankles, or arms.
* Hypoallergenic gloves must be provided for employees with allergies to standard gloves.
* Replace gloves as soon as possible after becoming contaminated, torn, punctured, or otherwise compromised.
* All skin defects such as cuts, abrasions, ulcers, areas of dermatitis, etc. should be covered with an occlusive bandage.
* PPE must be removed after it becomes contaminated and before leaving the work area.
* Disposable gloves may not be washed or decontaminated for re-use.
* PPE, including lab coats and gloves, must not be worn in public areas such as the bathrooms, elevators, break rooms or general office areas. It must be removed prior to leaving the work area.
* Masks in combination with eye protection devices, such as goggles or glasses with solid side shields, or chin length face shields, are required to be worn whenever splashes, spray, splatter, or droplets of blood or other potentially infectious materials may be generated and eye, nose, or mouth contamination can be reasonably anticipated, such as when transferring samples to an incubator or emptying waste traps.
* All disposable PPE must be discarded in biohazard waste containers and all biohazardous waste policies must be followed.

1. In a setting where there is a high risk of exposure to an agent that is contracted through aerosolization, employees will be required to wear an N95 respirator. Medical surveillance, a written respiratory protection plan, and fit testing/training must be conducted before personnel wear N95 respiratory protection. Contact RedMed to schedule fit testing. (Remove this item if not applicable.)
2. No use of headphones, cell phones, ear buds, etc., while in the BSL-2 lab.
3. No animals (pets) or minors (persons under the age of 18) will be allowed to enter the lab at any time.
4. Mouth pipetting is prohibited; mechanical pipetting devices are to be used at all times.
5. All procedures are to be performed carefully to minimize the creation of splashes or aerosols. Aerosols containing RG2/BSL-2 agents pose an increased risk of exposure to personnel. Solutions containing RG2/BSL-2 agents will be enclosed in capped containers during agitation (vortexing, shaking, centrifugation, etc.). If agitation is performed outside of a safety cabinet, two levels of containment are required (i.e. capped tube enclosed in plastic bag or Tupperware). After agitation, containers will only be opened in a biosafety cabinet to prevent exposure to personnel from aerosols. Human cells/tissue will be homogenized inside a biosafety cabinet. Centrifugation of hazardous materials must be performed using centrifuge safety caps or sealed rotors: these should be loaded and unloaded in the BSC.
6. Wipe work surfaces with an appropriate disinfectant (*name of disinfectant*) after experiments and immediately after spills. Follow SOP in Section H, Spill Response Procedures.
7. Decontaminate all contaminated or potentially contaminated materials by appropriate methods before disposal (refer to Section K, Disposal of Biohazardous Waste).
8. Follow all manufacturer’s instructions and SOPs when using any of the laboratory equipment.
9. Wash hands with soap (i) after handling materials involving organisms containing recombinant or synthetic nucleic acid molecules including bacteria and viruses, (ii) after handling animals, (iii) after working with human cells, (iv) after removing PPE, and (v) whenever exiting the laboratory.
10. All sharps must be handled with extreme caution. The clipping, breaking, and recapping of needles is not permitted. Plastic pipettes and pipette tips are considered puncture hazards and should be treated as sharps. See Section G for correct handling and disposal of sharps.
11. *Fluorescence activated cell sorting (FACS) can produce aerosols and special precautions must be used. BSL-2 samples that only require analysis must be fixed in 4% paraformaldehyde prior to running on a cell sorter whenever possible. For sorting BSL-2 cells, permission must first be obtained from the FACS operator prior to scheduling the run. The FACS operator must be informed about the type of cells being run and the hazards of the cells. Adapt the SOP found* [*here*](https://ibc.utah.edu/library.php)*. (Remove this item if not applicable).*
12. Transport of biological materials to another building or lab within the same building should be done in a sealed, leakproof container labeled with the universal biohazard sticker. If the samples are infectious, use a secondary container and label it with the contents and a contact person/phone number.
13. All cultures, stocks, and other regulated wastes are decontaminated by autoclaving or disinfection before disposal. **NOTE: No untreated or non-disinfected biological agent-containing material, including recombinant and synthetic nucleic acids, should be allowed into any drain connected to the sanitary sewer system (e.g., from a sink).** Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. See Section H for correct disposal of solid and liquid biohazardous waste.
14. Before exiting the lab, be sure that the equipment and work areas are clean, all contaminated waste materials are disposed of properly, and stocks have been returned to the proper storage area. Remove PPE and wash your hands.
15. **Laboratory-Specific Standard Operating Procedures**

This is the place to include the procedural details for your lab’s experiments with biological agents and toxins handled at BSL-2. If the SOPs include specialty equipment that requires training to operate safety, be sure to include those descriptions here. See Appendix 12 for sample SOPs.

1. **Laboratory Signage**
2. The universal biohazard symbol will be used to mark regulated waste containers, refrigerators containing biological agents, blood or Other Potentially Infectious Material (OPIM) contaminated equipment, specimen containers, and specimen transport containers. The universal biohazard symbol must be located at the entrance to all laboratory rooms where blood or OPIM is used. Red bags can be used as a substitute for bags marked with the universal biohazard symbol for regulated waste only.
3. Permanent yellow warning signs containing the biohazard symbol and BSL-2 designation are posted at the entrance to rooms where Risk Group 2 agents are used or stored. This system is intended for rooms where the use of BSL-2 containment is expected to continue over a long time period without frequent total room decontamination. This door sign outside of the laboratory is posted and managed by EHS. Biosafety level 2 labs should have the following permanently-affixed decals: Biohazard symbol, “BSL-2” designation, and Exit Requirements. The types of agents worked with in the lab are listed but not specifically identified. If any changes need to be made to the sign, including emergency contacts, please contact the Biosafety Office at [biosafety@ehs.utah.edu](mailto:biosafety@ehs.utah.edu). EHS will post any changes to the sign after IBC approval and completion of an EHS inspection.



1. **Spill Response Procedures**

All spills involving Recombinant DNA or Synthetic Nucleic Acid Molecules and hazardous biological materials should be cleaned up using appropriate biosafety procedures, described below. If there is any doubt about what to do, call the PI (*Telephone #*), or the Biosafety Office 1-6590, or the University’s internal emergency number: 5-2677.

**The following items should be included in a biological spill kit:**

* **Disinfectant** – Prepare a fresh 1:10 bleach solution. In other words, a pre-measured amount of bleach in a spray bottle is placed in the spill kit, but the cold water required to dilute the bleach is not added until right before use. Otherwise, use an [EPA-registered disinfectant](https://www.epa.gov/pesticide-registration/epas-registered-antimicrobial-products-effective-against-bloodborne) (effective against HIV and HBV) following manufacturer’s instructions. Examples are Cavicide, Cidex OPA and Clidox-S. Note the date of manufacture and/or expiration.
* **Absorbent material** (paper towel, absorbent powder)
* **Personal protective equipment** (e.g., disposable gloves (2 pairs), eye protection, face shield or surgical mask, lab coat, shoe covers). It is necessary to review the PPE in the spill kit on a regular basis to verify quality. Gloves can degrade over time. At the first sign of degradation (e.g., discoloration, brittleness, stickiness, tearing), replace the gloves in the spill kit with new ones. Likewise, the strap on splash goggles can undergo similar degradative processes.
* **Mechanical tools** (forceps or tongs, broom and dustpan) – Dispose in biohazardous waste after spill response. Purchase inexpensive plastic tools for this purpose.
* **Waste container** (biohazard bags) – By assembling all of the spill materials in a bucket or other leak-proof and puncture-proof container, you will have a secondary container readily available for proper containment of your biohazard bag.

1. **Spills inside of a Biosafety Cabinet**
   1. Stop work.
   2. If you are splashed by the material, change PPE. Always change gloves.
   3. Keep the biosafety cabinet running.
   4. Contain the spill by covering with paper towels (to avoid splashes or aerosols).
   5. Prepare the disinfectant*.*
   6. Saturate spill with XXXXXXX *(fill in the appropriate decontaminant).* Let sit for 20-minute exposure time.
      1. For large spills (greater than 10ml) use undiluted bleach or disinfectant.
      2. In the event of a spill into the drip pan/catch basin, add an equal volume of disinfectant and wait for 20 minutes to clean up the disinfected material.
      3. *Note: due to its evaporative nature alcohol, is not recommended as the primary disinfectant but can be used to remove bleach/disinfectant residue.*
      4. *If working with human blood or OPIM (such as human cell line) spills must be disinfected with an* [*EPA-approved disinfectant*](https://www.epa.gov/pesticide-registration/epas-registered-antimicrobial-products-effective-against-bloodborne)*.*
   7. Wipe up spill, disposing of towels in biohazard bag.
      1. If the biohazard bag is to be autoclaved, liquid bleach should be neutralized with sodium thiosulfate after it is used for disinfection. A good rule of thumb is if your absorbent towels are dripping wet, the bleach should be neutralized prior to autoclaving.
   8. Wipe or spray spill area with XXXXXXX *(fill in the appropriate decontaminant).* Allow to air dry.
   9. Disinfect all other materials used in the biosafety cabinet by disinfecting the surface with XXXXXXX *(fill in the appropriate decontaminant)* with a 20-minute contact time. Do not attempt to disinfect contaminated cardboard or other paper items that absorb liquid: contaminated items should be disposed of as solid biohazard waste.
   10. If bleach or other corrosive disinfectant used, wipe spill area and disinfected equipment with alcohol or water.
   11. Place all towels or absorbent materials into a designated container for biohazardous waste.
   12. Remove PPE, discard disposable PPE as biohazardous waste, and wash hands.
2. **Spills outside of a Biosafety Cabinet**
   1. Stop work.
   2. If you are splashed by the material, dispose of PPE and wash hands.
   3. Ensure that any other people in the vicinity are notified that a spill has occurred and that the room should be evacuated. Post a “Do Not Enter” notice on the door. Notify the PI or lab supervisor.
   4. If you need assistance with the spill clean-up, call EHS (1-6590).
   5. Wait 60 minutes before re-entering the room to allow aerosols to settle.
   6. Assemble Spill cleanup materials and don PPE, including lab coat, eye protection and face shield or mask, 2 pair of gloves, shoe covers. If the lab coat does not have cuffed sleeves, disposable sleeve covers should be worn.
   7. Contain the spill by covering with paper towels (to avoid splashes or aerosols).
   8. Saturate spill with XXXXXXX *(fill in the appropriate decontaminant).* Let sit for 20-minute exposure time.
      1. For large spills (greater than 10ml) use undiluted bleach or disinfectant.
      2. Wipe areas around the spill that may have splatter and any reusable equipment with XXXXXXX *(fill in the appropriate decontaminant).*
      3. If working with human blood or OPIM (such as human cell line) spills must be disinfected with an [EPA-approved disinfectant](https://www.epa.gov/pesticide-registration/epas-registered-antimicrobial-products-effective-against-bloodborne).
   9. Wipe up spill, disposing of towels in biohazard bag: if sharps may be present use tongs or a brush and pan and dispose in biohazard sharps container.
      1. Work concentrically to clean up the absorbent material. Always work from the outer edge of the spill toward the center.
      2. If the biohazard bag is to be autoclaved, liquid bleach should be neutralized with sodium thiosulfate after it is used for disinfection. A good rule of thumb is if your absorbent towels are dripping wet, the bleach should be neutralized prior to autoclaving.
   10. Wipe or spray spill area with XXXXXXX *(fill in the appropriate decontaminant).* Allow to air dry.
   11. If bleach or other corrosive disinfectant used, wipe spill area and disinfected equipment with alcohol or water.
   12. Remove PPE, discard disposable PPE as biohazardous waste, and wash hands.
   13. Remove the “Do Not Enter” sign, and inform others that it is safe to re-enter the room.
   14. Once the spill has been contained, complete an [EHS Incident Report.](https://oehs.utah.edu/incidentnear-miss-report)
3. **Spills Inside of a Centrifuge Contained Within a Closed Cup, Bucket, or Rotor**
   1. Put on lab coat, gloves, and proper eye protection prior to opening centrifuge. Open carefully to assess the damage.
   2. Prepare the disinfectant*: consult the instructions of the centrifuge rotor to identify suitable disinfectants*.
   3. If the spill is contained within a closed cup, bucket, or rotor, spray the exterior with disinfectant and allow at least 20 minutes of contact time. Remove the carrier to the nearest biosafety cabinet (BSC).
      1. *Note, if possible, avoid using bleach on centrifuge rotors and buckets to avoid damaging the equipment. If bleach is used, ensure all surfaces are wiped down with soap and water after disinfection. Alternatively, use an EPA-registered disinfectant, such as Cidex or Cavicide.*
   4. Gather supplies needed, such as a sharps container for broken glass and bins filled with disinfectant and place into the BSC.
   5. Open the centrifuge rotor or bucket inside of the BSC. Use a mechanical device (forceps, tongs, etc.) to remove broken glass and place directly into sharps container. Carefully remove any unbroken tubes and place into a bin filled with XXXXXXX (*fill in the appropriate decontaminant*) for at least 20 minutes. Wipe carrier/bucket with disinfectant.
   6. After disinfection, carrier, bucket, or rotor must be washed with a mild soap and water.
   7. Spray the interior of the centrifuge chamber with XXXXXXX (*fill in the appropriate decontaminant*), let sit for at least 20 minutes and then wipe down with soap and water.
   8. Dispose of all clean-up materials (except sharps) in an appropriate biohazardous waste container. Dispose of sharps in a biohazard sharps container.
   9. Remove PPE, discard disposable PPE as biohazardous waste, and wash hands.

If you are concerned that the spill is not contained within the rotor or bucket:

* + 1. Ensure that any other people in the vicinity are notified that a spill has occurred, and the room should be evacuated. Post a “Do Not Enter” notice on the door. Notify the PI or lab supervisor.
    2. If you need assistance with the spill clean-up, call EHS (801-581-6590).
    3. Wait 60 minutes before re-entering the room to allow aerosols to settle.
    4. Proceed with clean up as described above.

**Note**: Many centrifuge rotors can be disinfected by autoclaving. Check the manufacturer’s instructions.

1. **Emergency Spills: Environmental Risk**
   1. Stop work.
   2. Ensure that any other people in the vicinity are notified that a spill has occurred and that the room should be evacuated. Post a “Do Not Enter” notice on the door. Notify the PI or lab supervisor.
   3. Call EHS (801-581-6590). Provide information on the nature of the material spilled.
   4. Take appropriate precautions to limit exposure or spread of spill to other areas.
2. **Post-Exposure Response Procedures**

**Exposures include:**

* Direct skin, eye, or mucosal membrane exposure to biological agents.
* Parenteral inoculation by a syringe needle or other contaminated sharp (needlestick).
* Ingestion of liquid suspension of an infected material or by contaminated hand to mouth exposure.
* Inhalation of infectious aerosols.

**In the event of an exposure, follow these steps immediately:**

1. Stop work.
2. Remove exposed PPE, taking care to avoid contact of unexposed areas to infectious agents on the PPE.
3. Inform others in the immediate area about any biohazardous materials out of containment to prevent further exposure.
4. Immediately wash affected areas with soap and water, or if exposure to eyes or mucous membranes occurred, immediately flush affected area with water for 10-15 minutes.
5. For serious/life threatening exposures or chemical burns, call 911.
6. After washing, notify lab supervisor or Principal Investigator of the exposure if they are immediately available. If not, seek medical attention first and then report the exposure to them later.
7. Seek medical attention (if 911 has not already been called for serious/life threatening exposure).
   * Go immediately to the RedMed Employee Health Clinic at the University Union Building or the Occupational Medical Clinic at the Redwood Health Center. After 5pm you will be seen by an Urgent Care Physician at the Redwood Health Center. After 8:30pm, you may choose to seek medical attention the next morning if the exposure is not urgent. Alternatively, go to the University of Utah Hospital Emergency Department.
   * Some exposures require immediate medical attention in order to initiate post-exposure prophylaxis as soon as possible. Therefore, if after 8:30pm, you **MUST** go to the Emergency Department. These include exposures to:
     + Human samples from a patient (or animal) with a known infectious disease, such as HBV, HIV, Rabies, Invasive Group A Streptococcus, Invasive Meningococcus, Tuberculosis.
     + Samples from macaque or other old-world primates
     + A pathogenic agent or toxin for which post-exposure prophylaxis is available and must be started immediately:
       1. Herpes B virus
       2. Human Immunodeficiency virus
       3. Hepatitis B virus
       4. Varicella Zoster virus
       5. Rabies virus
       6. Recombinant lentiviral or retroviral vectors
       7. *Group A Streptococcus*
       8. Neisseria meningitidis
       9. *Mycobacterium tuberculosis*
       10. Pertussis Toxin
       11. Tetanus Toxin

* For those who find it more convenient, Occupational Medicine (~7:30a-12:30p) and an Emergency Department (24 hours) are available at South Jordan Health Center.

**RedMed Employee Health Clinic**

**(ground floor of the A. Ray Olpin Student Union Building)**

200 Central Campus Dr.

Salt Lake City, UT 84112

Phone: (801) 213-3303\*\*

Hours: M-TH: 8:00AM – 5:00PM, Friday: 9:00AM – 3:30PM

Closed 1:30PM-2PM

\*\*calling first is recommended, as this is a smaller clinic, and for some exposures/injuries, they may recommend Redwood Health Center.

**Redwood Health Center**

Occupational Medicine Clinic

1525 West 2100 South

Salt Lake City, UT 84119

Phone: (801) 213-9777

Hours: M-F 8:00AM – 5:00PM

**After Hours**

**Redwood Urgent Care**

1525 West 2100 South

Salt Lake City, UT 84119

(801) 213-9900

M-F 5:00PM – 8:30PM

Sat.-Sun.: 9:00AM – 8:30PM

**After 8:30 PM, wait until next day, or:**

Emergency Department at University Hospital

(main floor, northeast side of the hospital)

50 N. Medical Drive

Salt Lake City, UT 84132

(801) 581-2291

**An alternative Occ Med and ER location:**

**South Jordan Health Center**

5126 W. Daybreak Parkway

South Jordan, UT 84009

(801) 213-4500

1. Ensure that the physician is aware of all materials that were being used at the time of exposure. Also inform the Healthcare Provider of any medical conditions, such as pregnancy or immunosuppression, or drug treatment that you currently have or take.
2. **Post exposure prophylaxis must be initiated as soon as possible after exposure, if indicated.** Be sure to follow any physician-recommended follow-up evaluations or procedures.
3. Report the incident as soon as possible after medical care.
   * Ensure that the incident is immediately reported to the Biosafety Officer (801-581-6590 **AND** [biosafety@ehs.utah.edu](mailto:biosafety@ehs.utah.edu)) by the PI/Supervisor. If the project involves recombinant and synthetic nucleic acid molecules, the IBC will be required to report any significant problems with or violations of the [NIH Guidelines for Research with Recombinant or Synthetic Nucleic Acid Molecules](https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf) and any significant research-related accidents or illnesses to the NIH within 30 days.

**AND**

* + Fill out and submit the Employer’s First Report of Injury or Illness E1 Form 122. This form can be downloaded from the human resources website under “Forms > Absence Management” (<https://www.hr.utah.edu/forms/index.php>).
  + Note: Human resources will evaluate all incident reports to determine if cases meet OSHA’s Recordkeeping Requirements (29 CFR 1904).

**NOTE: Spill and Exposure Procedures must be clearly posted in the BSL-2 laboratory.**

1. **Use and Disposal of Sharps**

To prevent needle stick injuries:

* Do not recap needles by hand. RECAPPING OF NEEDLES IS PROHIBITED.
* Do not remove needles from syringes by hand.
* Do not bend, break, or otherwise manipulate needles by hand.
* Avoid using needles whenever possible.
* Replace glass materials with plastic (such as plastic Pasteur pipettes) whenever possible.
* Immediately after use, discard needle and syringe (whether contaminated or not) into puncture resistant sharps containers. RECAPPING OF NEEDLES IS PROHIBITED.
* Use a Food and Drug Administration (FDA)-cleared sharps container if you generate sharps waste (pictured below). A description of FDA-Cleared Sharps containers can be found [here](http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/HomeHealthandConsumer/ConsumerProducts/Sharps/ucm263236.htm). FDA-cleared sharps disposal containers are made from rigid plastic, come marked with a line that indicates when the container should be considered full, which means it is time to dispose of the container, and have the Universal Biohazard symbol.



* Never discard sharps into regular trash.
* Never discard sharps into bags of biological waste.
* Use care and caution when cleaning up after procedures that require the use of syringes and needles.
* Do not overfill sharps containers. Close completely when 3/4 full, request pickup from the EHS through the [SAM System](https://oehs.utah.edu/topics/lab-management-system).
* Locate sharps containers in areas in which needles are commonly used. Make containers easily accessible.
* Replacement sharps containers may be obtained through the [SAM System](https://oehs.utah.edu/topics/lab-management-system) or can be ordered from laboratory supply distributors, such as VWR and ThermoFisher. Be sure to select sharps containers that withstand autoclaving.

**Contaminated Serological Pipets and Pipet Tips**

Serological pipets (glass and plastic) and disposable pipet tips are considered puncture hazards and should be disposed of as sharps. Contaminated pipets and tips should be discarded in approved sharps containers, as described above.

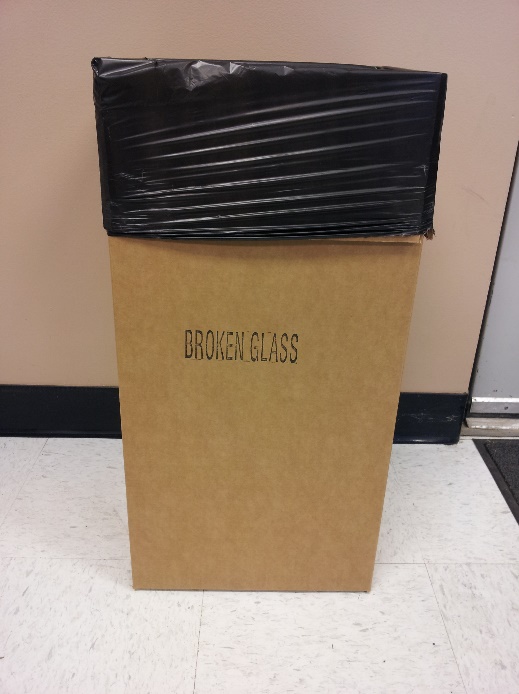
Due to the large size of serological pipets, investigators disposing of large numbers of these can request 20 or 44 gallon hard-sided biohazard waste containers (pictured below) from EHS through the [SAM System](https://oehs.utah.edu/topics/lab-management-system). These will be picked up by EHS staff as for other biohazardous waste.

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**44 Gallon Waste Container for Contaminated Serological Pipets and Pipet Tips**

**Decontaminated Serological Pipets and Pipet Tips**

It is possible to decontaminate serological pipets and tips prior to disposal. Ensure that both the inside and outside of the pipets or tips are exposed to the approved disinfectant (e.g., a freshly prepared 1:10 dilution of bleach) for at least 20 minutes. However, serological pipets and disposable tips are still considered puncture hazards. Therefore, after removing the disinfectant, they can be disposed of in a Broken Glass box (rigid puncture resistant boxes lined with a plastic bag and labeled “Broken Glass”: pictured below), which can be obtained from your custodial staff or from EHS. Once they are 3/4 full, they should be closed with tape and disposed of as regular trash by your custodians. **DO NOT dispose of CONTAMINATED SHARPS into the broken glass box!**



**Broken Glass Box**

**NOT for CONTAMINATED SHARPS!**

**Uncontaminated puncture hazards only!**

1. **Disposal of Biohazardous Waste**

All cultures, stocks, and other potentially infectious materials must be disinfected prior to disposal. This can be performed in the laboratory, or pick-up by EHS can be requested. Instructions for liquid and solid biohazardous waste are below.

* + 1. **Biowaste Disposal – Liquids**

Liquid waste generated from BSL-2 experiments, including aspirated tissue culture media, must be disinfected and then disposed. Bleach is typically used to disinfect liquids, but other agents, such as ZZZ, Vesphene III se, or other [EPA-approved disinfectants](https://www.epa.gov/pesticide-registration/epas-registered-antimicrobial-products-effective-against-bloodborne) may be used, if effective.

If you use bleach:

* Ensure the final concentration exceeds 0.5% sodium hypochlorite (no less than one part bleach to 9 parts liquid).
* Ensure the bleach is fresh. In tissue culture media traps, change at least twice weekly.
* Ensure the media is exposed to disinfectant for at least 20 minutes prior to disposal.
* Dispose down the sink.

If you use ZZZ or Vesphene III se:

* Ensure the final concentration exceeds 400ppm (one part disinfectant to 99 parts water).
* In tissue culture media traps, change at least every 3 months (indicate the date of the last change on the flask).
* Ensure the media is exposed to disinfectant for at least 20 minutes prior to disposal.
* Collect waste into containers marked “Unwanted Materials” and date when you start collecting. When full or 6 months after your start date (whichever happens first), arrange pickup by EHS through the SAM [website](https://oehs.utah.edu/topics/lab-management-system). **NO DRAIN DISPOSAL unless approved by EHS.**

If the container will be unattended (outside of your immediate control) then label it with the date, time, and the words “Biohazardous liquid,” and keep it in a secondary container (for example, a plastic tub) while it is disinfecting.

If you use other agents to decontaminate liquid cultures, follow the instructions on the packaging. Contact the Biosafety Office (801-581-6590) for advice on appropriate disinfectants and procedures for disposal of treated waste.

* + 1. **Biowaste Disposal - Solids**

Contaminated solid waste includes cloth, plastic, and paper items that have been exposed to agents infectious or hazardous to humans or animals, and shall be placed in a biohazard bag. During collection, the biohazard bag must be contained in a closeable, leak-proof container labeled with the biohazard symbol. Keep the container lid closed unless someone is working nearby and regularly adding waste to the container. When the biohazard bag is ¾ full, loosely tie or tape the bag closed. This waste can be autoclaved and disposed, or EHS can pick it up.

If you are autoclaving the waste:

* Place a Ziploc bag or balloon containing water in the bag when it is about half full to generate steam during autoclaving.
* The bag should be placed in a solid autoclave-resistant tray in the autoclave.
* After autoclaving, the waste can be disposed in the regular trash. Ensure that there is clear indication that the material has been autoclaved, such as by using autoclave tape. Prior to disposal, place the autoclaved biohazard bag into an opaque trash bag.

If requesting pick-up by EHS:

* Secure the lid on the waste container, and decontaminate the exterior with an appropriate disinfectant. Move it to a convenient securable storage location, or transport it to a biohazardous waste storage room, if available. Request a pickup from your lab using [SAM](https://sam.ehs.utah.edu/ehsa/).
* Replacement waste containers may be requested on SAM. They are solid sided, leak proof, lined with red biohazard bags, and labeled with a biohazard symbol.

1. **Laundry**

The University of Utah Hospital can be used to clean dirty lab coats and other articles that require laundering. Linen Services can be found in the Acute Care Building in the University Hospital, 801-581-2200. You must have a chartfield on-file with linen services for billing (~$2.20/lab coat). Alternatively, there are commercial laundry services that can clean contaminated lab coats, such as Cintas, Alsco, and Vestis.

The following laundering requirements must be met:

* Handle contaminated laundry as little as possible, with minimal agitation.
* Place wet contaminated laundry in leak-proof, labeled or color-coded containers before transport to the University Hospital Laundry.
* Contact outside providers for information on their transport requirements.

**Appendices**

The appendices are provided to supplement the material in this biosafety manual template. For any that are not relevant to your lab, they may be deleted from your biosafety manual. Much of the information on the safe operation of laboratory equipment contained in these appendices is based on [OSHA Fact Sheets](https://www.osha.gov/laboratories).

**Appendix 1: Conducting a Biological Hazard Risk Assessment**

In order to appropriately train lab personnel on the risks encountered in the laboratory, a risk assessment for the biological materials used in lab must first be conducted. See the full guidance document on risk assessments [here](https://ibc.utah.edu/_resources/documents/fact-sheets-and-sops/guielines-for-how-to-conduct-a-risk-assessment.pdf). Please include your risk assessment in Section C of this Biosafety Manual.

First, identify the intrinsic factors of the agent that contribute to the hazard. These include characteristics such as pathogenicity, virulence, and infectivity/communicability. Additional intrinsic factors include the natural mode of transmission, the infectious dose, and the potential impact of any genetic modifications performed in the lab.

Next, identify the laboratory procedures that could lead to an exposure event. Such procedures include:

* Aerosol generation (e.g., pipetting, mixing, blending, grinding, sonicating, vortexing, centrifuging, shaking)
* Manipulation with sharps
* Animal handling
* Contact with blood, bodily fluids, or other potentially infectious material
* Ingestion of agents via contaminated work areas
* Eye-splashes from liquid nitrogen storage

When performing a risk assessment of laboratory procedures, all potential routes of exposure should be addressed. Most laboratory-acquired infections have resulted from inhalation of aerosols, splashes or sprays, and needlesticks. It is good practice to look for potential exposures via ingestion, inoculation, inhalation, and contamination of skin and mucous membranes and attempt to identify safer alternatives and risk mitigation strategies.

**Appendix 2: Use of a Biosafety Cabinet (BSC)**

Remove Appendix if not applicable.

Laboratory personnel must be trained on appropriate use of the laboratory biosafety cabinet. The OSHA fact sheet can be found [here](https://www.osha.gov/sites/default/files/publications/OSHAfactsheet-laboratory-safety-biosafety-cabinets.pdf). The University of Utah fact sheet can be found [here](https://ibc.utah.edu/_resources/documents/fact-sheets-and-sops/biological-safety-cabinets-fact-sheet.pdf) and includes a description of the three classes of biosafety cabinets.

1. Don appropriate PPE (fluid resistant lab coat, gloves, eye protection).
2. Confirm that the BSC is currently certified for use. (Annual certifications are performed by ENV. Contact the Biosafety Office at [biosafety@ehs.utah.edu](mailto:biosafety@ehs.utah.edu) if the re-certification date listed on the certification sticker has passed).
3. Turn on the blower in the cabinet, and confirm it is working properly by checking the airflow gauges. Readings indicate relative pressure drop across the HEPA filter. Higher readings may indicate filter clogging. Zero readings may indicate loss of filter integrity. In either of these cases, notify the Laboratory Manager or PI and EHS. University of Utah Facilities Management does not perform maintenance on biological safety cabinets.
4. Adjust the stool height so that armpits are level with the bottom of the view screen or sash.
5. Prior to beginning work, the BSC should be decontaminated. Clean the inside surfaces of the BSC with *(name of disinfectant)* and follow with water or 70% ethanol (if using bleach or other EPA-approved disinfectant). DO NOT put head inside the cabinet. To reach the back of the cabinet use an extension, such as a Swiffer handle.
   * *Note: due to its evaporative nature, alcohol is not recommended as the primary disinfectant but can be used to wipe down previously disinfected surfaces or remove bleach/disinfectant residue.*
6. Prepare a written checklist of materials necessary for the experiment, and place only necessary materials in the BSC before beginning work. Allow the blower to operate for about 3 minutes to purge particulates before beginning work.
7. DO NOT disrupt the airflow through the hood by placing ANY item, including arms, on the grills. Work with both arms raised slightly. Minimize in/out arm movement, and when necessary, move arms in and out of the cabinet slowly, perpendicular to the face opening, to limit disruption of the air curtain.
   * Manipulation of materials inside the cabinet should be delayed for 1 minute after placing hands/arms inside the cabinet to allow the air to stabilize and to “air sweep” arms.
8. Avoid unnecessary clutter in the BSC. Large equipment should be avoided, or placed as far back as practical. Do not work with open containers of hazardous materials in front of the large equipment.
9. Organize the work surface for a clean-to-dirty work flow. Place clean pipets, flasks, and sterile media bottles at one side of the cabinet; place discard or kill pans containing disinfectant, biohazard waste containers, used flasks, spent cultures, and other waste on the other side.
10. While working, perform work at least 4 inches back from the front opening of the cabinet.
11. Use the aseptic techniques below to reduce splatter and aerosol generation:

* Opened bottles or tubes should not be held in a vertical position.
* Hold the lid above open sterile containers to minimize direct impact of downward air.
* Open flames CAN NOT be used because they create turbulence that disrupts the pattern of air supplied to the work surface.

1. After manipulating infectious agents, make sure all containers are tightly closed.
2. Plastic pipettes with a cotton plug shall be used for pipetting liquids containing biological materials. The electric pipettor shall be fitted with a 0.2 µm filter to prevent aerosol-based contamination.
3. A beaker or discard pan, containing a freshly prepared 1:10 solution of commercial bleach, shall be placed inside the biosafety cabinet during the cell culture work.
4. After pipetting liquid containing biological materials, the dilute bleach solution in the beaker shall be pipetted up and down the full length of the pipette or left in the pan. Serological pipettes and tips should be placed in a in a puncture resistant sharps container or other approved receptacle.
5. After decontamination, pipette tips shall be removed from the pipettor and temporarily left in the beaker containing bleach in the biosafety cabinet.
6. Small volumes of liquid waste containing biological materials shall be collected in a beaker containing undiluted bleach inside the biosafety cabinet. The final concentration of bleach should be at least 10% of the final volume (>0.5% sodium hypochlorite). After completing work, wait at least 20 minutes before disposing down the drain.
7. Large volumes should be collected by vacuum aspiration into a flask containing an appropriate disinfectant. Turn off the house vacuum when not in use. **NOTE: No untreated or non-disinfected biological agent-containing material should be allowed into any drain connected to the sanitary sewer system (e.g., from a sink).**

* *The flask should be placed in a secondary container to prevent it from tipping over and be labeled with a biohazard sticker. The vacuum line should be protected by an in-line 0.2 µM filter. The vacuum filters must be replaced if clogged or if liquid makes contact with the filter. Examples include Whatman Vacu-guard and Pall Gelman Vacushield in-line disk filters. Used filters should be placed in the biohazard waste.*

1. At the completion of the work, the beaker containing the plastic pipettes and tips shall be removed from the biosafety cabinet. Pipettes and tips shall be lifted out of the beaker, the bleach solution allowed to drain back into the beaker, and the pipettes and tips placed in a puncture resistant sharps container or other approved receptacle. NOTE plastic pipette tips and serological pipettes are treated as sharps.
2. At the completion of the work, all materials will be removed from the biosafety cabinet: all items must be surface decontaminated prior to removal. *Describe methods for decontamination.*
3. Clean the inside surfaces of the BSC with *(name of disinfectant)* after completion of work, and follow with 70% ethanol or water (if using bleach).

* *Note: alcohol should not be used as the primary disinfectant but can be used to remove bleach/disinfectant residue.*

1. The drip pan under the work surface must be cleaned at least monthly, or after spills, with *(name of disinfectant)*. If spilled liquid enters through the front or rear grilles, close the drain valves and pour decontaminating solution into the drip pan. Allow to sit for appropriate contact time.

**Notes of Caution:**

1. Many BSCs are equipped with UV lights for surface disinfection. UV lights must be turned off before work begins in the hood. **Do not look directly at UV lights as this can cause eye damage**.

* *UV light is effective only for disinfecting clean, solid surfaces with which it comes in contact. It is not effective in decontaminating the cabinet air flow. UV light is not effective against bacterial spores. UV germicidal light tubes should be replaced frequently (at least every 6 months for biosafety cabinets in use on a daily basis) to assure that they are emitting light at 254 nm and at an intensity appropriate for decontamination. Due to concerns over the effectiveness of these lights and the risks to individuals in the room, some Institutions, such as the NIH, have banned their use in BSCs. The University of Utah IBC strongly recommends that UV lights are not relied on as a method of decontamination: see Fact Sheet* [*here*](https://d2vxd53ymoe6ju.cloudfront.net/wp-content/uploads/sites/4/20180725083508/UV-Lamps-in-Biological-Safety-Cabinets-Fact-Sheet.pdf)*.*

1. Any use of volatile solvents, such as absolute ethanol, should be kept to a minimum or done elsewhere. **Dangerously high levels of volatile vapors can accumulate inside the cabinet and pose a threat of fire or explosion.**

**Appendix 3: Use of an Autoclave**

Remove Appendix if not applicable.

Autoclaves can be used to sterilize biohazardous materials before releasing to the normal waste systems (sanitary sewer for liquids, permitted landfill for solid waste). Their use for treating infectious waste is regulated by Utah State Law, [Rule R315-316](https://adminrules.utah.gov/public/rule/R315-316/Current%20Rules?). Laboratory personnel must be trained on appropriate use of the autoclave. The OSHA fact sheet can be found [here](https://www.osha.gov/sites/default/files/publications/OSHAquickfacts-lab-safety-autoclaves-sterilizers.pdf). The University of Utah SOP can be found [here](https://ibc.utah.edu/_resources/documents/fact-sheets-and-sops/autoclave_use_and_testing_sop_09_27_21.docx.pdf).

1. Appropriate PPE is required for use, including standard laboratory PPE as well as a heat-resistant glove (such as an oven mitt) for handling hot items.
2. There is a potential for burns when operating an autoclave, both from contact with the autoclave itself and the items inside and from steam leaving the apparatus. Do not remove items from an autoclave until they have cooled.
3. If you are autoclaving sharp instruments, use forceps or other tools to remove them from the autoclave.
4. The operator shall have available, and shall certify in writing that they understand, written operating procedures for each steam sterilizer, including time, temperature, pressure, type of waste, type of container, closure of container, pattern of loading, water content, and maximum load quantity. Be sure to read and follow recommendations made by the manufacturer in the owner’s manual.
5. Infectious waste shall be subjected to sufficient temperature, pressure, and time to inactivate *Bacillus stearothermophilus* spores in the center of the waste load at a 6 Log10 reduction or greater. Unless a steam sterilizer is equipped to continuously monitor and record temperature and pressure during the entire length of each sterilization cycle, each package of infectious waste to be sterilized shall have a temperature-sensitive tape or equivalent test material, such as chemical indicators, attached that will indicate if the sterilization temperature and pressure have been reached. Understand the temperature and pressure readings recorded by the autoclave reflect chamber conditions, not necessarily the conditions achieved in the waste material.
6. Autoclaves must be tested weekly or after every 40 hours of use, whichever is longer. The Biosafety Office provides the testing materials and will pick them up for processing. Contact [biosafety@ehs.utah.edu](mailto:biosafety@ehs.utah.edu) for assistance.
7. A written log of autoclave use must be maintained including date, time, operator name, amount and type of waste, temperature, pressure, and length of cycle.
8. Use only those types of containers, bags, and lids that are designed for autoclaving. Inspect vessels for cracks or chips. Make sure to use biohazardous waste bags that are rated for autoclave use.
9. If autoclaving dry biohazardous waste, add a glove or Ziploc bag containing ~50 mL of water to the autoclave bag to help produce steam inside the bag.
10. Prior to loading the autoclave, visually inspect the drain strainer to ensure it is clean.
11. Place labware and waste bags in a secondary container (typically a steel bin).
12. Arrange loads to allow free circulation of steam. Do not overfill the autoclave.
13. Select the appropriate autoclave setting for the materials being autoclaved. Recommended settings for dry waste are at least 121 °C, 15-17 psi, for at least 30 min.
14. DO NOT autoclave flammable or corrosive liquids, including bleach. It can damage the autoclave and release hazardous gases. If small amounts of bleach need to be autoclaved, it can first be neutralized with sodium thiosulfate.
15. DO NOT autoclave radioactive materials.
16. DO NOT place loose sharps in autoclave bags. Place sharps in sharps waste containers, and submit a pickup request for these in [SAM](https://sam.ehs.utah.edu/ehsa/). DO NOT autoclave the sharps waste containers.
17. Once the waste has been sterilized as shown by the affixed indicator tape, place the biohazardous waste bag in an opaque trash bag for regular trash disposal.

**Appendix 4: Use of Centrifuges**

Remove Appendix if not applicable.

Centrifuges operate at high speed and have potential for causing bodily harm if not used properly. Unbalanced centrifuge rotors can cause centrifuges to fail, and sample container breakage can release harmful aerosols. Lab personnel must be properly trained in the use of the specific centrifuges for their work. The majority of all centrifuge accidents result from user error. The OSHA fact sheet can be found [here](https://www.osha.gov/sites/default/files/publications/OSHAquickfacts-lab-safety-centrifuges.pdf).

1. Inspect the centrifuge prior to use. Make sure rotor compartments are dry, that the interior is clean, and that the rotor is seated properly.
2. Care for the O-rings as instructed by the centrifuge user manual.
3. Inspect your sample containers prior to use. They should be dry, free of cracks or flaws, not overfilled, and sealed.
4. Only use matching tubes, buckets, and other equipment. Make sure the samples in the rotor are balanced.
5. Always use centrifuge safety cups to contain potential spills and aerosols.
6. DO NOT exceed the rotor’s maximum run speed.
7. Close the centrifuge lid prior to starting the centrifuge.
8. Before leaving the area, make sure the centrifuge has safely reached operating speed.
9. Make sure the rotor has come to a complete stop before opening the centrifuge.
10. When centrifuging infectious materials, wait several minutes before opening the lid. If there is a spill or breakage during centrifugation, stop the centrifuge. If it is suspected or possible that the spill is not contained by the safety cups, all personnel should evacuate the room for at least 60 minutes. Upon re-entry personnel should don PPE, including eye and face protection. After opening the centrifuge, buckets should be transferred to the BSC. The centrifuge and buckets should be disinfected by cleaning with (*disinfectant*) and then wiped down with 70% ethanol.

**Appendix 5: Safe Handling of Cryogenic Liquids and Dry Ice**

Remove Appendix if not applicable.

Cryogens are substances, such as liquid nitrogen (LN2), used to produce very low temperature. Although not a cryogen, solid carbon dioxide (dry ice) is also commonly used in the laboratory. Proper procedures are required to avoid hazards posed by cryogens or dry ice, such as burns and asphyxiation. The OSHA fact sheet can be found [here](https://www.osha.gov/sites/default/files/publications/OSHAquickfacts-lab-safety-cryogens-dryice.pdf).

**General Precautions when working with dry ice or LN2:**

* Avoid eye or skin contact. Never handle with bare hands, and always use eye protection. Use tongs to handle dry ice.
* Use cryogenic gloves when manipulating samples and cryogens. These gloves need to be loose-fitting so they can be readily removed if LN2 or dry ice gets into them.
* Never store LN2 or dry ice in confined areas such as cold rooms. Do not transport in an elevator. In these cases, an oxygen-deficient atmosphere can result and lead to asphyxiation. When removing dry ice from a storage cooler, do not “lean in” to the cooler, as it will have an oxygen-deficient atmosphere.
* Never store LN2 or dry ice in a sealed, airtight container. The pressure resulting from the production of gaseous carbon dioxide or nitrogen may lead to an explosion.
* Cryogenic sample vials immersed in LN2 have the potential to explode. Wear face and eye protection when manipulating them. They are designed to be used in the vapor phase.
* In case of exposure, remove any clothing that is not frozen to the skin. DO NOT rub frozen skin, because it can result in tissue damage. Seek medical attention.
* Place the affected skin in a warm water bath (not above 40 °C or 104 °F). DO NOT use dry heat.

**Appendix 6. Plasmid Maps**

Remove appendix if not applicable.

*Insert images and/or descriptions of plasmid maps of: 1) viral packaging and transfer plasmids, 2) gene editing plasmids, 3) transgenic pathogen plasmids, 4) plasmids including biological toxins.*

**Appendix 7: Gene Editing Questionnaire**

For all protocols involving gene editing, such as CRISPR/Cas9, complete the following questionnaire. Remove appendix if you are NOT using gene editing.

1. Please specify which gene editing technology is being used (CRISPR/Cas9, Zinc Finger nucleases, TALENS, Meganucleases, other?).
2. How is the gene editing technology being delivered to cells (e.g., nanoparticles, plasmid transfection, lentivirus, adeno-associated virus, etc.)?
3. What are the gene targets for these experiments?
4. Are any of the targets oncogenes, tumor suppressors, or genes involved in cell cycle regulation?
5. Are you targeting embryos or germ line cells?
6. Are you planning research using gene editing technologies in humans, human embryos, or human germ cells?

**NOTE:** No gene editing of the germ line, human embryos or germ cells for clinical application is allowed. Gene editing of human embryos and germ cells for scientific purpose may be allowed, but must be evaluated on a case-by-case basis by the appropriate federal and local scientific review committees.)

1. Please identify the cell types, tissues and/or organisms that are being targeted.

**Answer the following questions for studies involving CRISPR/Cas9:**

1. Are the guide RNA (gRNA) and nuclease encoded on the same plasmid, viral vector, or other delivery vehicle? If yes, describe.
2. For gene editing research involving viral vectors, have potential off target effects by your gRNA been identified in the human genome? This is helpful in assessing the risk of potential exposure in the event of an incident. The IBC recommends GT Scan (<http://bioinformatics.csiro.au/gt-scan/>), but other utilities may be used. Please identify which utility you have used and provide a list of off target genes (with up to 3 mismatches).
3. Does the construct encoding either Cas9 or gRNA also contain DNA with homology to genomic DNA that flanks the gRNA target site? If so, answer the questions below.
   1. Will the experiment introduce this construct into the germ line of an organism?
   2. Will the experiment result in a transgenic, sexually reproducing organisms?
   3. If the answer is yes to both 10a and 10b, provide a complete description of your experiment.

**Answer the following questions for studies involving Gene Drives:**

1. Are you performing a “gene drive” experiment?

“Gene drive” is defined as a technology whereby a particular heritable element biases inheritance in its favor, resulting in the heritable element becoming more prevalent than predicted by Mendelian laws of inheritance in a population over successive generations. Per the 2024 iteration of the NIH Guidelines, Section III-D-8, experiments involving gene drive modified organisms generated by recombinant or synthetic nucleic acid molecules shall be conducted at a minimum of BSL-2, ABSL-2 (Animals) or BSL2-P (plant) containment.

If Yes, please edit Section B of the Biosafety Manual to include a Risk Assessment that addresses each of the following:

* The specific types of manipulations based on
  + Function or intended function of the genetic/gene drive construct (i.e., a designed or engineered assembly of sequences)
  + Source of the genetic material (e.g., sequences of transgenes) in the construct
  + The modifications to the construct
  + Whether it is possible to predict the consequences of a construct, including the recognition of the potential to introduce an unintended gene drive (i.e., construct not specifically designed as a gene drive but nonetheless having properties of a gene drive) into the organism and the possible consequences of escape into the environment
  + The potential ability of the gene drive to spread or persist in local populations
* The types of scientific questions that need to be answered and what data are needed to facilitate the risk assessment
* Options for approaches to risk mitigation for specific risks in experiments or when dealing with a high degree of uncertainty about risks
* When to consider implementation of more stringent containment measures until sufficient biosafety data are accrued to support conduct of the research at a lower containment level.

Appendix 8: **Important Information for Working with Human Samples and Cells**

Remove Appendix if not applicable.

Minimum Personal Protective Equipment:

* 1. Face protection (goggles, face shield or other splatter guard) is used to protect from potential aerosols, splashes or sprays of infectious or other hazardous materials to the face when cells must be taken outside the BSC (e.g., for microscopy).
  2. Protective laboratory coats or gowns, designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
  3. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

Potential Hazards:

The potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV, HCV and HIV, as well as agents such as Mycobacterium tuberculosis that may be present in human lung tissues. Potential hazards to laboratory workers are presented by cells transformed with viral agents, such as SV-40, EBV, or HBV, as well as cells carrying viral genomic material. Tumorigenic human cells also are potential hazards as a result of self-inoculation.

Procedure:

Human cells should be handled using BIOSAFETY LEVEL 2 practices. All work should be performed in a biosafety cabinet or using splash protection, and all material must be decontaminated by autoclaving or disinfection before discarding. All employees working with human cells and tissues must complete annual Bloodborne Pathogens training, and must work under the policies and guidelines established by the institutions' Exposure Control Plan.

**Appendix 9: Sharps Injury log**

Remove appendix if not applicable (i.e., if the lab does not use sharps).

##### Laboratory Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Year\_\_\_\_\_

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Date | Type of Device (e.g. syringe, suture needle) | Brand Name of Device | Work Area where injury occurred (e.g. Geriatrics, Lab) | Brief description of how incident occurred (i.e., procedure being done, action being performed (disposal, injection, etc.), body part injured) |
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29 CRF 1910.1030, OSHA’s Bloodborne Pathogens Standard, in paragraph (h)(5), requires an employer to establish and maintain a Sharps Injury Log for recording all percutaneous injuries in a facility occurring from contaminated sharps. The Log must be retained for five years following the end of the year to which it relates. The Log must be kept in a manner that preserves the confidentiality of the affected employee.

**Appendix 10: Annual Review of Safe Sharp Devices**

Remove appendix if not applicable (i.e., if the lab does not use sharps)

According to the Needlestick Safety and Prevention Act (Pub. L. 106-430), all laboratories working with blood or OPIM and with sharps (e.g., needles, syringes with needles, scalpels, capillary tubes, and lancets) must complete and document an annual review of the availability of safer sharps alternatives such as needleless systems and sharps with engineered protections. If safer viable alternatives are identified they must be adopted.

This form (or equivalent) must be completed on an annual basis by any University of Utah Facility/laboratory that performs sharps-related procedures on human samples or other potentially infectious material.

Reviewer’s Name: Job Title:

Department/Clinic: Date:

Supervisor/PI Name: Telephone #:

The purpose of this form is to document:

* Sharps devices currently in use;
* The criteria used in the selection of the safer sharps devices in use, and;
* Annual consideration of new safer sharps devices.

Complete the table below as completely as possible to document the sharps devices that are being used. Use multiple pages if necessary. **This review form must be maintained with your safety records.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Device #1** | **Device #2** | **Device #3** |
| **Name of Sharps Device** |  |  |  |
| **Manufacturer** |  |  |  |
| **Model/Size in Use** |  |  |  |
| **Procedures Performed** |  |  |  |
| **\*Safer Sharps Device?**  **(Y/N)** |  |  |  |
| **Description of Safety Feature** |  |  |  |
| **Justification for Selection (must consider newly marketed safer sharps devices) or why a safer sharp cannot be used** |  |  |  |

\*A justification must be documented for any device that does **not** meet the criteria of a safer sharps device (see *Sharps with engineered sharps injury protection* definition below). Acceptable justifications include, but are not limited to:

* Use of a safer sharps device will jeopardize patient or employee safety.
* Use of a safer sharps device is medically inadvisable.
* Market unavailability of an appropriate safer sharps device.

**NOTE:** Cost is not typically an acceptable justification.

**Sharps with engineered sharps injury protection:** This includes non-needle sharps or needle devices containing built-in safety features that are used for collecting fluids or administering medications or other fluids, or other procedures involving the risk of sharps injury. This description covers a broad array of devices, including:

* Syringes with a sliding sheath that shields the attached needle after use;
* Needles that retract into a syringe after use;
* Shielded or retracting catheters
* Intravenous medication (IV) delivery systems that use a catheter port with a needle housed in a protective covering.

**Appendix 11: Biological Toxin SOP**

Remove appendix if not applicable.

*You must complete this appendix if the lab works with acute biological toxins. Complete one for each type of toxin. Included are any toxins on the select agent list and/or with an LD50 less than 100 µg/kg body weight. This includes, but is not limited to, botulinum toxins, tetanus toxin, pertussis toxin, diphtheria toxin, tetrodotoxin, shiga toxin, and conotoxins.*

*[Customize text in parentheses and brackets to specific procedures and equipment in your laboratory]. Please refer to the* [*Biosafety in Microbiological and Biomedical Laboratories*](https://www.cdc.gov/labs/bmbl/index.html)*, Appendix I: Guidelines for work with Toxin of Biological Origin for more information.]*

|  |  |
| --- | --- |
| Standard Operating Procedures for *[Toxin]* | |
| #1 Chemicals/Hazards | [*Obtain specific toxin hazard information from MSDS/SDS*.*]*  CAS number: *[XXX]*  Routes of exposure: *[XXX]*  How exposure might occur: *[XXX]*  Target organs: *[XXX]*  Signs/symptoms of exposure: *[XXX]* |
| #2 Prior to Work | Hazardous chemical and specific SOP training will be provided to personnel working with toxin and any other personnel authorized or required to be in the laboratory during toxin work.  Appropriate inactivation method(s) for [*toxin*] will be determined and supplies for inactivation and spill cleanup of *[toxin]* will be readily available.  *[List vaccinations or antitoxins required or recommended for toxin]*. |
| #3 Environmental/  Ventilation Controls | Work with *[toxin]* will be performed in a *[chemical fume hood/Biological Safety cabinet (BSC)]*.  *List the type of BSC to be used (e.g. Class II, Type A2).*  In-line HEPA filters will be used on vacuum lines.  Safety centrifuge cups or sealed rotors will be used if centrifuging materials containing *[toxin]*, and the outside surfaces will be routinely decontaminated after each use. |
| #4 Personal Protective Equipment (PPE) | The following PPE will be worn when working with *[toxin]*: *[Customize list]*   * Laboratory coat or gown with long cuffed sleeves * Disposable lab coat * Disposable sleeves * Safety glasses with side shields or chemical safety goggles * Face protection such as a face shield if splash/spatter possible * Gloves *[type]* that are impervious to *[toxin]* and diluent * Respiratory protection *[if aerosol hazard is present] If respirators are used the worker must be enrolled in the Respiratory protection program; contact EHS for information (*[*http://d2vxd53ymoe6ju.cloudfront.net/wp-content/uploads/sites/4/20160922155338/Resp-Prot-Program-rev.2016.pdf*](http://d2vxd53ymoe6ju.cloudfront.net/wp-content/uploads/sites/4/20160922155338/Resp-Prot-Program-rev.2016.pdf)*).*   Gloves must be changed immediately if contaminated, torn, or punctured. |
| #5 Special Handling Procedures & Storage Requirements | **HANDLING**  Prep   * Sign will be posted on the room door when toxin is in use stating: “Toxins in Use -- Authorized Personnel Only.” * All preparation of *[toxin]* will be performed over plastic-backed absorbent pads in a *[fume hood/BSC]*. Pads will be disposed of immediately upon contamination and after completion of tasks. * Describe how toxin will be prepared*: [Example: Vials of [toxin] will be purchased in pre-weighed powder form and then reconstituted in a [fume hood/biological safety cabinet (BSC)]. Weighing the [toxin] is not necessary as reconstitution will occur in the purchased vial and then aliquoted into vials with caps.]*   Use   * Only needle locking (Luer-Lock type) syringes or disposable syringe units will be used for injection or aspiration of *[toxin]*. * A sharps container will be in the immediate vicinity for safe sharps disposal. * Containers will be decontaminated before they are removed from *[fume hood/BSC]*. * The *[fume hood/BSC]* will be decontaminated upon completion of tasks with *[decontaminant and concentration]* for *[contact time]*. * All potentially contaminated disposable items will be placed in a hazardous waste bag and decontaminated before disposal. * Hands will be washed upon completion of tasks.   **STORAGE**   * *[Toxin]* will be stored in locked *[freezer/refrigerator/cabinet/box/other]* in *[secure location room #]*.   **TRANSPORT**   * *[Toxin]* will be transported in labeled and sealed non-breakable secondary containers. |
| #6 Spill and Accident Procedures  *[Specific cleaning, decontamination agents (and contact times)/equipment and waste disposal procedures must be determined.]* | All spills will be cleaned by properly protected and trained personnel only. Wash hands thoroughly after completing any spill clean-up. If you are not trained or comfortable cleaning up a spill, call EHS for assistance at 801-581-6590. If it is an emergency (risk of exposure to others such as an on-going toxin release), call 911.  **Liquid spills:**  Personnel cleaning up a liquid spill will wear a lab coat/gown with cuffed sleeves (or disposable sleeves), goggles, and two pairs of nitrile gloves. Cover spill with absorbent paper towels and apply *[inactivating agent + concentration]*, starting at the perimeter and working towards the center, allowing *[XX min]* contact time to deactivate *[toxin]*. Clean the spill area with *[inactivating agent]*, then soap and water. The decontaminated spill waste will be double bagged and disposed of in the biohazard waste container.  **Powder spills inside of [*fume hood/BSC]*:**  Personnel cleaning up a powder spill will wear a lab coat/gown with cuffed sleeves (or disposable sleeves), goggles, and two pairs of nitrile gloves. Gently cover powder spill with dampened absorbent paper towels to avoid raising dust. Apply *[inactivating agent + concentration]*, starting at the perimeter and working towards the center, allowing *[XX min]* contact time to deactivate *[toxin]*. Clean the spill area with *[inactivating agent]*, then soap and water. The decontaminated spill waste will be double bagged and disposed of in the biohazard waste container.  **Powder spills outside of a *[fume hood/BSC]*:**  Remove all personnel from the room and restrict access; do not attempt to clean up the spill unless personnel are authorized to use a respirator. If personnel are not cleared to use a respirator, report the spill by notifying EHS (at 801-581-6590). Tell them that a spill has occurred, and you need EHS to assist with the spill cleanup.  Be prepared to provide the following information:   * Name and phone number of knowledgeable person that can be contacted: *[emergency contact name and phone number]* * *[Toxin name]*, concentration and amount spilled, liquid or solid spill * Number of injured, if any * Location of spill   This information can also be used in reporting to the Emergency Department after potential exposure.  Personnel cleaning up a powder spill will wear a lab coat/gown with cuffed sleeves [*or disposable sleeves*], goggles, two pairs of nitrile gloves and a respirator. Gently cover powder spill with dampened absorbent paper towels to avoid raising dust. Apply *[inactivating agent + concentration]*, starting at the perimeter and working towards the center, allowing *[XX min]* contact time to deactivate *[toxin]*. Clean the spill area with *[inactivating agent]*, then soap and water. The decontaminated spill waste will be double bagged and disposed of in the biohazard waste container.  For questions on spill cleanup, contact EHS at 801-581-6590 for guidance.  Wash hands thoroughly after completing any spill clean-up. |
| **EXPOSURE PROCEDURES**  **In Case of Emergency** | Follow the post-exposure procedures found in Section I of this Biosafety Manual. |
| #7 Waste Disposal and Cleaning | Any waste *[toxin]* will be decontaminated or autoclaved as appropriate before disposal or given to EHS for disposal whenever possible.  Work space surfaces must be wiped down after completion of tasks with *[inactivating agent + concentration]* during the length of the experiment. Absorbent pads will be replaced after completion of tasks or immediately if contaminated. Used and potentially contaminated absorbent pads, PPE, etc. will be placed in a hazardous waste bag and autoclaved.  If in-lab inactivation is not possible for *[toxin]* waste, it must be managed as hazardous chemical waste. Be aware that some form of treatment in the lab may be required before it can be managed as chemical waste. Contact EHS at 801-581-6590 for disposal instructions. For chemical waste pick up complete a request through the lab management system (<http://oehs.utah.edu/topics/lab-management-system>). |
| #8 Special Precautions for Use of *[Toxin]* in Animals  *(This section must be completed if working with toxin in animals)*  *Identify where animals will be injected, housed and any special precautions/warnings for animal handlers.* | Use of toxins in animals will be documented and approved by IACUC.  *[Give detailed procedures for safely completing tasks, containment, decontamination information, and any special disposal requirements.]*  *[Animals will be anesthetized or placed into a restraining apparatus before procedures using [toxin] are performed. Once the animal has been properly fitted into the restraining apparatus, the syringe will be loaded just prior to injection.]*  After procedures are complete, the restraining apparatus and surrounding work station will be decontaminated *[inactivating agent + concentration]*. All reusable lab equipment will be autoclaved.  *[Give any special disposal requirements]* |
| #9 Approval Required | The protocol must be approved by the Institutional Biosafety Committee prior to commencement. All staff working with *[toxin]* must be trained on this SOP prior to starting work. They must also be trained on the *[toxin]* MSDS/SDS, and it must be readily available in the laboratory. All training must be documented and maintained by the PI. |
| #10 Decontamination | All surfaces will be decontaminated with *[inactivating agent + concentration]* after removing the plastic backed pads. All reusable lab equipment will be autoclaved. Note that some disinfecting agents may not deactivate *[toxin]*. |
| #11 Designated Area | All work with [*toxin*] must be done in a designated laboratory, work space and *[fume hood/BSC]*. Signage must be placed on door to room when *[toxin]* is used. This work will be conducted in *[Room #]* |

Appendix 12: **Sample SOPs**

Remove after providing your own SOPs in section F of the manual.

These protocols are an example of a comprehensive SOP for generating and using recombinant lentiviral particles. It is provided as an example of a well-written and thorough SOP. Please remove this, but provide similar detail in your lab-specific SOPs in Section F of this manual.

1. *Containment:*

All manipulations will be performed in a type II Biological Safety Cabinet at BSL2 levels of containment.

The following personal protective equipment (PPE) will be required:

* Disposable or onsite-laundered lab coat: if sleeve cuffs are not tight, disposable sleeve covers must be worn.
* Gloves
* Safety glasses or goggles for work outside of the BSC, such as transporting materials, emptying traps, etc.
* Long pants and closed shoes

1. *Preparation of Concentrated Lentiviral Particles*

1. Expand HEK 293T cells in DMEM media supplemented with 10% FBS (heat inactivated) in 10 cm tissue culture dishes. The cells are cultured at 37 °C in 5% CO2.

*For unknown reasons, high passage HEK 293T cells do not produce high titer lentivirus. It is best to obtain a fresh stock of HEK 293T from ATCC rather than acquire stocks from other laboratories. Also, cryopreserve early passage cells and thaw a fresh stock for the generation of high titer lentivirus.*

1. Aspirate the media and wash each plate with 5 ml of PBS.
2. Add 1 ml/plate of trypsin and incubate for 2-5 min. Gently pipette up and down to mix the trypsin solution.
3. Add 10 ml/plate of DMEM media supplemented with 10% FBS (heat inactivated) and determine the number of cells per ml using a hemocytometer.
4. Plate 4-7 x 106 cells into each 10 cm tissue culture dish and adjust the volume of each plate to 10 ml with DMEM media supplemented with 10% FBS (heat inactivated). Culture the cells overnight at 37°C in 5% CO2.

*Alternatively, a confluent 10cm plate of HEK293T cells can be re-plated at a 1:10 dilution and cultured for 2-5 days until they are approximately 90% confluent. A typical procedure utilizing 24 10cm plates yields approximately 0.9-1.2 ml of a solution containing between 5.0 x107 to 5.0 x108 infectious units/ml (IU/ml).*

1. Aspirate the media and add 8 ml/plate of DMEM media supplemented with 10% FBS (heat inactivated) that has been pre-warmed to 37°C and culture for 2 h.
2. Prepare PEI at 1 mg/ml (w/v) in deionized sterile water.

*The transfection procedure described in this protocol is optimized for PEI (Huh et al., 2007). The PEI solutions can be aliquoted into 1.5 ml microcentrifuge tubes and stored at -20*°C *for up to a year. If a different transfection reagent will be used, optimization will be necessary.*

1. Add 720 µl of the 1 mg/ml (w/v) PEI solution to 11.28 ml of OPTI-MEM media in a 50 ml conical tube, which yields a solution sufficient to transfect 24 plates (30 µg of PEI per plate in 500 µl of OPTI-MEM).

*The volume can be adjusted depending on the number of plates utilized.*

1. Prepare a solution of plasmids in a separate 15 ml conical tube, which is enough to transfect 24 plates, by adding 120 µg of pHIV-Luc-ZsGreen (5 µg/plate), 40.8 µg of pMDLg/pRRE (1.7 µg/plate), 40.8 µg of pRSV-Rev (1.7 µg/plate), and 40.8 µg of pCMV-VSV-G (1.7 µg/plate) to a final volume of 12 ml of OPTI-MEM media (500 µI/plate).

*It is important to utilize plasmid solutions at >0.5 µg/µl since lower concentrations can dilute the media/DNA solution and decrease the transfection efficiency. In addition, to achieve high viral titers, it is necessary to mix all four plasmids together before mixing with the PEI solution.*

1. Add 12 ml of the plasmids/OPTI-MEM to the 12 ml of PEI/OPTI-MEM and mix. Incubate the solution for 30 min at room temperature.
2. Add 1 ml/plate dropwise of the PEI and plasmid OPTI-MEM solution, gently swirl the plates and culture cells with solution for 24 h at 37 °C in 5% C02.
3. Every time before working with solutions containing virus, place a large beaker containing >50 ml of bleach in a biosafety cabinet to disinfect any media and pipettes (make sure the final concentration of bleach is >10% before removing it from the biosafety cabinet).

*Appropriate personal protection equipment are required. Tubes containing virus can only be opened in a biosafety cabinet. Sealed tubes must he sprayed with 70% ethanol solution before removal from the biosafety cabinet.*

1. Remove the media from each plate and transfer it to the beaker containing bleach. Add 8 ml/plate of DMEM media containing 10% FBS (heat inactivated) that has been pre-warmed to 37°C and incubate overnight at 37°C in 5% CO2*.*
2. 48 h post transfection, remove the media from each plate and transfer it to a 50 ml conical tube on ice. Immediately add 8 ml/plate of fresh pre-warmed DMEM media containing 10% FBS (heat inactivated) to each plate and culture overnight at 37 °C in 5% CO2.

*The transfection efficiency can be estimated at this stage by using a tissue culture microscope with fluorescence capability and observing the percent of cells fluorescent for Zsgreen. It is advisable that this is performed on an extra plate of cells that are fixed with 4% paraformaldehyde, to inactivate the lentivirus, prior to microscopy. Continue with the concentration procedure only if* >*50% of the cells are fluorescent. If <50% of the cells are transfected, optimize the transfection procedure before proceeding.*

1. Centrifuge the 50 ml conical tubes containing the media for 10 min at 2,500 x g at 4°C to pellet any large debris.
2. Pass the supernatant through a 0.45 µm cellulose acetate filter and transfer 32 ml (about 4 plates worth) to each of the six ultracentrifuge tubes. Place tubes in titanium buckets on ice. Balance bucket pairs by adjusting the weight with DMEM media (this is done in the biosafety cabinet until the buckets are sealed).
3. To pellet the virus, ultra-centrifuge the solution for 1 h 45 min at 112,000 x g at 4 °C using a swinging bucket rotor.
4. Carefully remove the supernatant and transfer it to a beaker containing bleach. Immediately (do not let the pellet dry) add 150 µl of DMEM/F12 media to each centrifuge tube on ice. Gently pipette up and down without creating bubbles to dissolve the pellet. Place the ultracentrifuge tubes in a 50 ml conical tube and cap the conical tube to prevent evaporation of the solution. Store the conical tubes at 4 °C overnight.
5. The following day, collect the second round of media from the plates (72 h post transfection) and process it in the same manner as steps 14-17.

*Add bleach at a final concentration of 10% to the plates, incubate at room temperature for 20 minutes in the biosafety cabinet, and discard plates and media.*

1. After the ultracentrifugation step 17, remove the supernatant. Resuspend the pellets by transferring the DMEM/F12 solution containing the concentrated virus that was collected 48 h post transfection to each tube. Gently pipette up and down to dissolve the pellet. Place the ultracentrifuge tubes in a 50 ml conical vial to prevent evaporation and incubate at 4 °C for 2 h to ensure the virus is completely dissolved.
2. Pipette up and down to resuspend the pellets, being careful to limit bubbles. Combine all of the resuspended pellets into one tube and gently pipet to mix. Aliquot 50-100 µl of the concentrated virus into 500 µl microfuge tubes and store at -80 °C.

*The virus solutions are aliquoted to minimize freeze/thaws, which can significantly reduce the number of IU/ml. The virus solutions can be stored for up to 1 year at -80* °C.

1. Decontaminate all materials in the Biosafety Cabinet prior to removal from the cabinet and decontaminate the cabinet as described in Section E.1 of this manual.
2. Remove all PPE. Discard disposable PPE as biohazardous waste and disinfect safety glasses.
3. Wash hands prior to leaving the BSL-2 suite.
4. *Determining Infectious Units (IU)lml by FACS*
   1. All manipulations will be performed in a type II Biological Safety Cabinet at BSL2 levels of containment. See Section E.1 of this manual for detailed instructions on the use of a BSC.
   2. The following personal protective equipment (PPE) will be required:
      * *Lab coat.*
      * *Gloves*
      * *Safety glasses or goggles for work outside of the BSC, such as transporting materials, emptying traps, etc.*
      * *Long pants and closed shoes*
   3. Expand HEK 293T cells in DMEM media supplemented with 10% FBS (heat inactivated) in 10 cm tissue culture dishes.
   4. Aspirate the media and wash each plate with 5 ml of PBS.
   5. Add 1 ml/plate of trypsin and incubate for 2-5 min at 37°C in 5% CO2. Gently pipette up and down to mix the trypsin solution.
   6. Add 10 ml /plate of DMEM supplemented with 10% FBS (heat inactivated) and determine the number of cells per ml using a hemocytometer.

5. Plate 2.0 x 105 cells in each well of a 6 well tissue culture plate and adjust the volume to 3 ml/well with DMEM media supplemented with 10% FBS (heat inactivated ). Culture the cells overnight at 37 °C in 5% CO2*.*

6. Aspirate the media from four wells of the 6 well plate and ad d 1 ml/ well of DMEM media supplemented with 10% FBS ( heat inactivated) that has been pre-warmed to 37°C.

7. Aspirate the media from the remaining two wells of the 6 well plate and carefully wash with 2 ml/well of PBS so the cells do not detach.

8. Add 0.5 ml of trypsin to each of the two wells and incubate for 2-5 min at 37 °C in 5% CO2. Gently pipette up and down to mix the trypsin solution.

1. Add 0.5 ml/well of DMEM media supplemented with 1 0% FBS and determine the number of cells present in each well independently using a hemocytometer. Calculate the average number of cells present in each of the two wells. This number will be used to determine the IU/ml.
2. Thaw an aliquot of concentrated virus in the biosafety cabinet.
3. Dilute the virus solution 1:10 in DMEM medium supplemented with 10% FBS (heat inactivated). For example, add 8 µl of virus solution to 72 µl of media.
4. Add different amounts of the diluted virus to each of the remaining four wells as described below, gently mix and culture overnight.
   1. No virus control
   2. 1 µl of diluted virus (1:10,000 final dilution)
   3. 10 µI of diluted virus (1: 1,000 final dilution)

d) 50 µI of diluted virus (1:200 final dilution)

*The final dilution values are based upon the use of a 1:10 initial dilution of the virus and 1 ml of media in each well. Different dilutions are required since a sample containing <15% ZsGreen positive cells determined by FACS is required to more accurately calculate the IU/ml.*

1. Remove the media 24 h post infection and aspirate into bleach. Add 3 ml/well of DMEM media supplemented with 10% FBS that has been pre-warmed to 37°C and culture for 2 additional days.
2. Remove the media 72 h post infection and aspirate into bleach. Carefully wash the wells with 2 ml/well of PBS so the cells do no detach.
3. Add 0.5 ml/well of trypsin and incubate for 2-5 min. Gently pipette up and down to mix the trypsin solution.
4. Transfer the cell suspension to a 15 ml conical tube on ice. Wash each well with 1 ml of HBSS containing 2% FBS and transfer this solution to the respective 15 ml conical vial.
5. Add paraformaldehyde to a final concentration of 2% to each tube and incubate at 4 °C for 20 minutes to inactivate any residual virus that may be present.
6. Centrifuge the cells for 5 min at 400 x g at 4 °C.
7. Aspirate the supernatant and carefully resuspend the pellet with 1 ml of HBSS containing 2% FBS
8. Centrifuge the cells for 5 min at 400 x g at 4 °C.
9. Aspirate the supernatant and carefully resuspend the pellet with 250 µl of HBSS containing 2% FBS
10. Pass the cell suspension through a 100 µm cell strainer and transfer to a FACS tube
11. Setup the gates and detector voltages, and analyze samples using the sequential gates described below.
    1. SSC vs. FSC: eliminate debris and gate only single cells
    2. FLl (ZsGreen) histogram: determine percent ZsGreen positive cells
12. Calculate the IU/ml using the formula below.

IU/ml = (average # of cells/well) \* (% ZsGreen positive cells/100) \*(virus dilution)

*The average* # *of cells/well was determined above in step 9. We typically use a dilution where the cells are <15% Zs Green positive. Titers ranging from 5 x 107 to 5 x 108 IU/ml are commonly achieved using this protocol.*

1. Decontaminate all materials in the Biosafety Cabinet prior to removal from the cabinet and decontaminate the cabinet as described in Section E.1 of this manual.
2. Remove all PPE. Discard disposable PPE as biohazardous waste and disinfect safety glasses.
3. Wash hands prior to leaving the BSL-2 suite.
4. *Transduction of cells*
   1. All manipulations will be performed in a type II Biological Safety Cabinet at BSL2 levels of containment. See Section E.1 of this manual for detailed instructions on the use of a BSC.
   2. The following personal protective equipment (PPE) will be required:
      * *Lab coat.*
      * *Gloves*
      * *Safety glasses or goggles for work outside of the BSC, such as transporting materials, emptying traps, etc.*
      * *Long pants and closed shoes*
   3. Seed 250,000 cells per well in a 6-well plate in 0.5 ml of medium.

*For suspension infections, use ultra-low attachment plates, and for monolayer infections, use regular tissue culture-treated plates. The best method of infection must be determined empirically for each tumor. For monolayer infections, allow the cells to attach for 24-48 hr prior to adding virus.*

* 1. Add polybrene to a final concentration of 1-4 µg/ml. The actual concentration should be empirically determined using the target cells. Add concentrated lentivirus using a multiplicity of infection (MOI) of 5-20 viral particles/target cell. Add additional medium to bring the final volume to 1 ml per well.

*The multiplicity of infection may also have to be optimized for different cells.*

* 1. Replace the virus-containing medium with fresh medium after overnight incubation.
  2. Remove the virus medium and add 2 ml of fresh medium.
  3. The cells should be handled using BSL-2 procedures until at least 72 hours has passed following infection and medium has been exchanged using at least five washes (50-Fold dilution with each wash).
  4. Incubate at 37°C with 5% CO2. Expression of virus proteins (e.g. ZsGreen) should be detectable by fluorescence microscopy after 72 hours. Feed the cells every day by exchanging the medium. Expand the cells until a sufficient number are obtained for transplantation.
  5. Decontaminate all materials in the Biosafety Cabinet prior to removal from the cabinet and decontaminate the cabinet as described on Page XX of this manual.
  6. Remove all PPE. Discard disposable PPE as biohazardous waste and disinfect safety glasses.
  7. Wash hands prior to leaving the BSL-2 suite.

1. *Transplantation of lentivirus-transduced cells into mice*
   1. The following personal protective equipment (PPE) are required:
      * *Lab coat.*
      * *Gloves*
      * *Safety glasses or goggles for work outside of the BSC, such as transporting materials, emptying traps, etc.*
      * *Long pants and closed shoes*
   2. Transduced cells should be considered potentially infectious and handled under BSL-2 procedures until they have undergone at least five washes (50-Fold dilution with each wash).
   3. Transduced cells will not be transplanted until Step 1 is performed and at least 72 hours has passed following the infection procedure.
   4. Cells should be transplanted under ABSL1 conditions following procedures approved by IACUC and by the IBC.
      * Cell lines that have not have been established to be [free of adventitious agents](https://ibc.utah.edu/_resources/documents/fact-sheets-and-sops/injection-of-human-cell-linesu-ibc-sop_2022.pdf) will require ABSL-2 containment.
2. *Testing Lentiviral Vector Stocks for Replication Competent Virus*
3. Harvest fresh vector-infected supernatant (vector stock)
4. Clear cells from growth medium by low-speed centrifugation.
5. Filter medium through 0.45 µm-pore-size cellulose acetate filters.
6. Transduce SupT1\* lymphocytes
   1. Plate SupT1\* cells into a 6 well plate at 105 cells/well in presence of 8 µg/ml of Polybrene (to enhance virus detection) and add 2 ml of RPMI-1640 Medium supplemented with 10% FBS (heat inactivated).
   2. Infect SupT1\* cells with serial dilutions of vector stock (starting with > 1 mL of unconcentrated supernatant or concentrated equivalent)
7. Grow SupT1\* cells for 3 weeks or longer (passage as necessary)
   1. Harvest supernatant as per above in Step 1.
   2. Perform ELISA assay for p24 antigen.
   3. Record results for each batch (expressed as infectious units/mL).