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###### University of Utah

**Biosafety Manual and Standard Operating Procedures**

**Biosafety Level 1 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-1 laboratories at the University of Utah. 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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**Laboratory Personnel Signatures and Acknowledgement of Review**

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 determined by the risk assessment: <https://ibc.utah.edu/training.php>.

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| **Name** | **Signature** | **Date** |
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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*:**

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**University of Utah**

**Biosafety Level 1 Standard Operating Procedures**

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* Remove appendices that are not relevant to your lab. Edit the others, as applicable to your work. Make sure to update this page (Table of Contents) to list the incorporated appendices. The IBC will review this information.

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6. **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-1 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).

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

# All personnel who work in the laboratory must receive adequate instruction from their supervisor prior to beginning work, with refresher training according to the indicated schedule. The requirements for qualification to work in the (*name of PI*) BSL-1 lab are:

1. 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.
2. Initial hands-on training by the PI or Lab Manager.
3. 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.
4. (*List any additional training required for your lab.)*

*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/).

For more information concerning training, also see the University of Utah Biosafety Manual on the IBC web site (<https://ibc.utah.edu/biosafety-policies.php>).

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

# Work with biological materials 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. 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.
2. 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.

* 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.
* 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.
* 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.
* All disposable PPE must be discarded in biohazard waste containers and all biohazardous waste policies must be followed.

1. All skin defects such as cuts, abrasions, ulcers, areas of dermatitis, etc. should be covered with an occlusive bandage.
2. No use of headphones and/or cell phones/iPODs etc., while in the lab.
3. No animals (pets) will be allowed in 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.
6. Wipe work surfaces with an appropriate disinfectant (*name of disinfectant*) after experiments and immediately after spill cleanup. Follow SOP in Section E, Spill Response Procedures.
7. Decontaminate all contaminated or potentially contaminated materials by appropriate methods before disposal (refer to Section H, 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 removing PPE, and (iv) 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. 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.
12. 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.
13. 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.
14. **Laboratory-Specific Standard Operating Procedures**

This is the place to include the procedural details for your lab’s experiments with biological agents handled at BSL-1. If the SOPs include specialty equipment that requires training to operate safety, be sure to include those descriptions here.

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 due to exposure to UV or fluorescent lighting, temperature extremes, and the effects of 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 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 8:30 pm you may choose to seek medical attention the next morning if the exposure is not urgent. Alternatively, after 8:30 pm, go to the University of Utah Hospital Emergency Department.
   * 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-1 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.

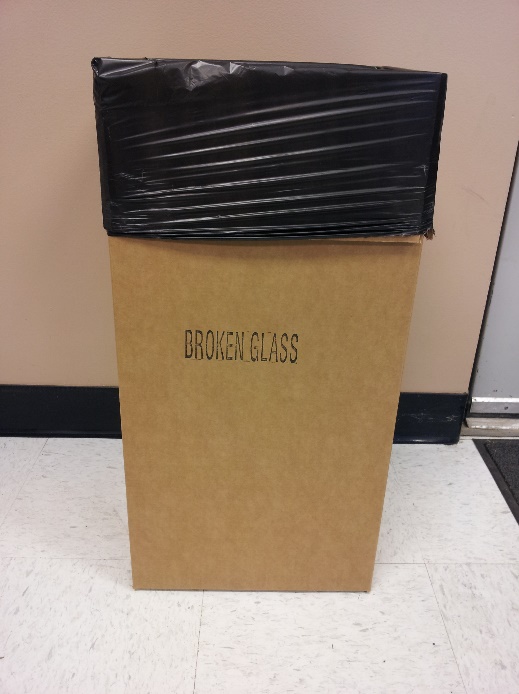
Due to the large size of serological pipets, investigators disposing of large numbers of these can request 20 or 44 gallon hard-sided biohazard waste containers (pictured below) from EHS through the [SAM System](https://oehs.utah.edu/topics/lab-management-system). These will be picked up by EHS staff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

Per the [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules](https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf), all contaminated liquid or solid wastes are decontaminated before disposal, including materials handled at BSL-1.

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

Liquid waste generated from BSL-1 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).

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.