

BSL1 and 2 Lab Specific Biosafety Manual Template



PURPOSE:

This Lab Specific Biosafety Manual should be prepared to provide the laboratory personnel at your lab with the information necessary to protect them and the surrounding environment from hazards associated with the use of biological materials in your lab. The guidelines which follow should provide a means for evaluating the risks of work involving biological materials and introduce the proper handling practices that will assist to minimize the risk of an occupational acquired infection. History has shown that if not handled appropriately, infectious agents can be transmitted to laboratory employees, and rarely, to people outside of the laboratory.

All Lab personnel working in your lab should read, understand, and sign this document as part of their training process when they first join your lab. Maintain an updated copy of this electronically or hard copy with the PI or Lab Manager. Document should be updated whenever there is a major change in the procedure or agents used in the lab.

**Submit a copy of this with your IBC New Protocol Registration Form for the committee to review.**

PI Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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**Text in RED and sections that do not apply to your lab should be edited or deleted! Comments should also be deleted prior to submission, including this one.**

# I. INTRODUCTION AND DESCRIPTION OF RESEARCH

Text in RED is meant to be modified by the PI.

Insert description of research. This should be thorough (i.e., not just one or two sentences!)

**Biological agents/organisms used:**

(Include bacterial, viral, fungal, recombinant, human/non-human primate unfixed tissues or cells)

*List organisms/biohazards here*

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| --- | --- | --- | --- | --- | --- | --- |
| **Type of Biological Material** | **Genetically Modified?** | **Insert (attach diagrams or link below)** | **Location(s) Used or Stored** | **Quantity (mg/ml/****vials)** | **Pathogen** | **Drug resistance with potential for increased pathogenicity?** |
| **Human** | **Animal** | **Plant** | **N/A** |  |
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# II. LABORATORY SIGNAGE

Laboratory signage should be up-to-date with all biological and chemical hazards, with current PI and emergency contacts. Warning signs containing the biohazard symbol and biosafety level designation are posted at the entrance to rooms. PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory or animal room must be included. The specific agents worked with in the lab do not need to be listed. If any changes need to be made to the sign, including emergency contacts, please contact RMS at askRMS@unt.edu

# III. USE OF BIOLOGICAL SAFETY CABINET

The biosafety cabinet is the primary means of protecting the researcher, the product, and the environment from biological hazards. All work with infectious agents should be manipulated in the BSC, especially those practices which could generate aerosols. Using the BSC properly includes the following (Training from ASU may be found here <https://youtu.be/q_C6xq7j-kg>):

* 1. Turn off the ultraviolet lamp if one is in use. Turn on the fluorescent lamp.
	2. Make sure the biological safety cabinet is certified.
	3. Inspect the air intake grilles for obstructions and foreign material and remove if necessary
	4. Turn on cabinet fan at least 15 minutes before beginning work, if not left running
	5. Don appropriate PPE. (Rear-fastening, long-sleeved gown with tight-fitting cuffs, safety glasses and a pair (or two pairs) of high quality nitrile gloves.)
	6. Disinfect work surface with an appropriate EPA registered disinfectant.
	7. Place items into the BSC, at least 6 inches from the front grill and approximately 2-4 inches from the rear grill, without unnecessary disruption of the airflow. (Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet.)
	8. Items used for surface decontamination and cleanup of a small spill should be included inside the BSC. Ensure there are biohazard waste containers inside of the BSC.
	9. Adjust the working height of the stool so that the worker's face is above the front opening.
	10. Work as far to the back (beyond the air split) of the BSC workspace as possible.
	11. Minimize the movement (e.g., sweeping) of arms and reduce the frequency of placing hands/arms into the BSC and taking them out
	12. Employ good microbiological practices, work with materials from the clean to the dirty side.
	13. Always use mechanical pipetting aids.
	14. Avoid using open flames inside BSCs. Flames disrupt the airflow and contribute to the heat load inside the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSCs.
	15. Do not work in a BSC while a warning light or alarm is signaling.
	16. Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper airflow and the level of protection provided.
	17. Keep the front and rear grilles clear.
	18. When work is completed, remove equipment and supplies from the cabinet. Wipe the bottom and side surfaces with disinfectant and allow cabinet to run for 5 minutes.
	19. Some BSCs are equipped with ultraviolet (UV) lights. If one is used, the tube should be wiped with 70% ethanol every two weeks, while turned off, to remove dust. UV radiation should not take the place of disinfectant for disinfection of the cabinet interior.
	20. The UV lamp should never be on while an operator is working in the cabinet.
	21. Minimize traffic around the biosafety cabinet and avoid drafts from doors and air conditioning.
	22. The BSC is certified annually by a contractor. To arrange for certification, contact BSO at biosafety@unt.edu
	23. When using the house vacuum system, place a hydrophobic filter (C) between overflow flask (B) and vacuum port (D). Examples include Whatman Vacu-guard and Pall Gelman Vacushield in-line disk filters. Turn off the vacuum when not in use.

**NOTES:** Be very careful when using small pieces of materials such as paper tissues in the hood. These can be blown into the hood and disrupt the motor operations. **Open flames are NOT permitted for use in BSCs**.

**Proper aspiration vacuum flask set up (**required in BSL2 labs**)**



1. Primary flask – used to collect liquid
2. Secondary flask (overfill flask) minimizes splash
3. In line filter between secondary flask and vacuum source ([FisherSci](https://www.fishersci.com/us/en/home.html%22%20%5Ct%20%22_blank) 09-744-75)
4. Vacuum line that is occasionally serviced by lab workers or UNT support personnel

# IV. USE AND DISPOSAL OF SHARPS

To prevent needle stick injuries:

* Avoid using needles whenever possible.
* Replace glass materials with plastic (such as Pasteur pipettes)
* Do not bend, break, or otherwise manipulate needles by hand.
* Do not recap needles by hand. Do not remove needles from syringes by hand.
* Immediately after use, discard needle and syringe (whether contaminated or not) into puncture resistant sharps containers. RECAPPING OF NEEDLES IS PROHIBITED.
* 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, and autoclave per the instructions in the UNT Biosafety Manual
* Locate sharps containers in areas in which needles are commonly used. Make containers easily accessible.

Sharps containers may be purchased from the supply stores on campus, as well as from laboratory supply distributors such as VWR and Fisher Scientific. Be sure to select sharps containers that withstand autoclaving.

In the event of a needle stick injury:

Wash the area thoroughly with soap and water. Notify supervisor immediately, fill out an [Incident Report form](https://riskmanagement.unt.edu/sites/default/files/incident_report_form.pdf) and submit to RMS, and report to the Student Health Center. Submit a Biohazard Incident Report form to biosafety@unt.edu within 48h.

# VI. SPILL RESPONSE PROCEDURES

Use the guidelines below for response to spills of biological materials outside of the biosafety cabinet. **(You MUST post your procedure within the lab)**

A biological spill kit is available in the laboratory. The kit contains [name and concentration of disinfectant], a package/roll of paper towels, autoclavable bags, protective eyewear and gloves [list type/kind], and forceps to pick up broken glass. The following individuals will be notified of the incident: [list here]. If the spill can be managed by laboratory personnel, proper personal protective equipment, including [list here], will be worn to clean up the spill.

A spill kit is to be kept in each area where work with biohazards is conducted. Spill kit contents should include: disinfectant (a dilute bleach solution or organism-specific disinfectant), paper towels, gloves, autoclave bags, sharps container, forceps (to pick up broken glass), and a broom and dustpan. A wrap-around laboratory coat and safety glasses should also be available in the kit. **Note:** A 1:10 dilution of household bleach and water (1 part bleach to 9 parts water), prepared fresh, is effective in most situations. Contact RMS Biosafety/Biosecurity for more information about the selection of disinfectants, particularly for any organisms suspected of being atypical in their sensitivity to disinfectants.

Immediate Action:

* Alert others in immediate area of the incident; notify the PI as soon as possible. If cleanup assistance is needed, contact RMS Monday-Friday, 8:00 a.m.- 5:00 p.m. or the UNT Police or local law enforcement agency non-emergency number listed on the contacts page for after-hour or weekends. Dial 911 if the spill constitutes an emergency.
* Put on gloves, safety glasses, and lab coat. If splashing is likely, wear goggles, face shield, and/or N95 respirator.
* Cover spilled material with paper towels and carefully pour an appropriate disinfectant onto paper towels in sufficient quantity to ensure effective microbial inactivation, proceeding from the outer edge of the spill to its center. Allow a 20-minute contact time to allow the disinfectant to inactivate the material.
* If broken glass or other sharps are present, use forceps to pick up item and discard into SHARPS container.
* Remove paper towels and other materials and dispose in biohazard waste container.
* Re-wipe spill area with disinfectant diluted to working strength. Wipe down any contaminated stationary equipment or furniture with disinfectant.
* Decontaminate (using an autoclave or approved chemical treatment method) reusable cleanup items and other reusable equipment. Do not autoclave bleach or other hazardous materials.
* Wash hands with soap and water.
* Notify laboratory personnel and the PI when the cleanup is completed.
* Complete the Biohazard Incident Report form and submit to the Biosafety Officer at IBCprogram@unt.edu and RMS at biosafety@unt.edu. If there were injuries, an [Incident report Form](https://riskmanagement.unt.edu/sites/default/files/incident_report_form.pdf) must also be submitted to RMS.

# VII. WASTE DISPOSAL PROCEDURES

# Contact Risk Management Services (940.565.2109 or askRMS@UNT.edu)

All biological waste in the research area will be handled and disposed of in accordance with federal, state, and local regulations as well as University policy. Materials containing recombinant DNA, synthetic nucleic acids, and genetically altered living organisms and their products are considered biohazardous waste. All biohazard bins should (i) Closable; (ii) made of material that can be easily disinfected (iii) Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping; (iv) Labeled or color-coded with biohazard symbol, lines with an biohazard red bag. Any materials to be decontaminated outside of the research area will be transported in a durable, leak-proof, closed container.

All personnel are responsible for maintaining a clean work area. Only trained individuals should operate the autoclave.

1. **Solid biological waste**: A spore-tested steam autoclave will be used to decontaminate the waste or waste will be collected in biohazard boxes and picked up by RMS (select all that apply).

[ ]  Solid biological waste will be collected in a bin with lid, labelled with biohazard symbol and lined with red bag. Once it is 2/3rd full the red bag should be tied with a knot and place in the cardboard biohazard box provided by RMS and the requested for picked up by RMS (biosafety@unt.edu) and disposed of by UNT’s biohazardous waste vendor. The cardboard box should not weigh more than 40 lbs. during pickup by RMS. You can place more than one tied red bag per cardboard box.

[ ]  Solid biological waste will be collected in a red, autoclavable bag, in secondary containment, and autoclaved in a spore-tested steam autoclave for 70 minutes at 123°C. Records will be kept for 3 years for autoclave spore testing, autoclave cycles, and waste amounts for 3 years for review by the State (TCEQ). Visit the <https://riskmanagement.unt.edu/forms-and-templates> for more details.

[ ]  Solid human and animal waste may require special handling procedures. Please contact RMS to discuss further.

1. **Liquid biological waste:** An appropriate chemically compatible disinfectant or a spore-tested steam autoclave will be used to decontaminate the waste (select all that apply).

[ ]  An appropriate disinfectant (e.g., bleach, Lysol, Virex) will be added to the liquid biological waste to obtain the final concentration (e.g., 1:10, 1:25) and contact time per manufacturer’s guidelines. **List disinfectant, concentration, and contact time:**

[ ]  A spore-tested steam autoclave will be used to disinfect the liquid biological waste (only if bleach or other hazardous chemicals are NOT present in the solution). Records will be kept for 3 years for autoclave spore testing, autoclave cycles, and waste amounts for 3 years for review by the State (TCEQ).

**List autoclave location and cycle parameters**:

After decontamination, the liquid waste is considered chemical waste. Submit an online [Hazardous waste-pickup request](https://riskmanagement.unt.edu/riskman/index.php?section=hazmatpickup) or drain dispose if pre-approved by RMS. Contact Risk Management Services (940.565.2109 or askRMS@UNT.edu) to request drain disposal approval. **List RMS approval date**:

1. Uncontaminated waste

Uncontaminated non-sharp waste should be disposed of in the general lab waste stream.

Uncontaminated broken glass is disposed of in a sturdy cardboard box, preferably lined with a plastic bag. When full, the box should be taped closed and disposed of in the dumpster. Housekeeping will not dispose of broken glass.

1. Sharps disposal

Sharps are items which pose a puncture or cutting hazard, such as glass, needles, and razors. Sharps should be disposed of in approved autoclave-resistant puncture-proof containers. Please refer to section IV of this manual for more information.

1. Animal Carcasses

Place animal carcasses/tissues into a plastic bag. Double bag all carcasses when zoonotic agents are present. Store bag in freezer until removal. Disposal of animal carcasses/tissues is coordinated through RMS.

# VIII. EMERGENCY PROCEDURES

1. Fire evacuation procedures

During a fire emergency, lab staff should prioritize life safety. Cultures and animals may be put away if time allows; if not, walk to the nearest exit. Pull the fire alarm if necessary, and call 911 once outside the building.

1. Power outage

In the event of a power outage, put away cultures and animals. Remove PPE and exit the lab normally. Emergency lighting within the buildings should provide adequate visibility to exit the building. Notify the PI immediately.

1. Medical emergency

In the event of a medical emergency in the lab, follow appropriate procedures depending on the hazards present. If the emergency involves a spill of hazardous agent onto the clothing or body, assist the victim to the shower or eyewash station. If the victim requires medical attention, call 911.

1. Accidental exposure or needlestick

For splashes to the eyes, rinse the eyes under the eyewash for 15 minutes. If there has been a needlestick, wash the affected area thoroughly with soap and water, then report to the Student Health Center or contact RMS for information on obtaining medical care.

In the event of an injury, an [incident report form](https://riskmanagement.unt.edu/sites/default/files/incident_report_form.pdf) must be filled out within 24h and submitted to RMS. In all cases of biohazard releases and incidents, a biohazard incident report form must be submitted to the BSO at IBCProgram@unt.edu and RMS at biosafety@unt.edu within 48h.

# EMERGENCY CONTACTS

**EMERGENCIES 911**

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Office Phone # | Home/Cell Phone # | Email |
| Principal Investigator  |  |  |  |
| Backup Contact  |  |  |  |
| Facilities Management Work Control Center | 940.565.2700 |  | Work.control@unt.edu |
| Ambulance/Fire/Police | 911 |  |  |
| Non-emergency UNT Police | 940.565.3000 |  |  |
| RMS | 940.565.2109 |  | askRMS@unt.edu |
| Biosafety Officer, Dr. Veena Naik | 940-369-7260 |   | veena.naik@unt.edu |
| Radiation Safety Officer, Fatima Adeyemo | 940.565.3282 |  | Fatima.Adeyemo@unt.edu |
| RMS for Waste Pick-Up |  |  | Biosafety @unt.edu |
| Student Health & Wellness Center | 940.565.2333 |  |  |
| Urgent Care Clinic |  |  |  |

# VIII POST EXPOSURE FACT SHEET

Example ONLY-delete and replace with **your** agents/chemicals

**Post-Exposure Procedures:**

***Salmonella typhimiruim***

**Characteristics:** Family Enterobacteriaceae; gram negative rod; motile, aerobic and facultatively anaerobic; serological identification of somatic and flagellar antigens; over 2000 serotypes capable of causing disease.

**Incubation Period:** Six to 72 hours, usually about 12-36 hours

**Symptoms:** Salmonellosis is an acute gastroenteritis; acute infectious disease with sudden onset of abdominal pain, diarrhea, nausea and vomiting; may progress to more serious septicemia, includes focal infections, abscesses, endocarditis, pneumonia; may also cause typhoid like enteric fever; some cases develop reactive arthritis (Reiter's syndrome) which may become chronic

**Infectious Dose:** 100 - 1,000 organisms - ingestion; varies with multiple factors

**What is a potential exposure?** Ingestion, needlestick or cut with contaminated sharp object, splash to the eye, contact with broken skin.

**Post-Exposure Treatment:** Skin exposure / Percutaneous: Wash affected area and apply antiseptic (3% H2O2), report to the Student Health Center/CareNow facility. Mucous membrane exposure (splash to eye): flush eyes for 15 minutes using eyewash, then report to Student Health Center/CareNow facility. Ingestion: Report to the Student Health Center/CareNow facility. Antibiotic therapy may be required.

**If symptoms appear with no known incidence of exposure:** Seek medical attention and inform the health care provider of the microorganisms used in the workplace.

**Prevention:** Biosafety level 2 practices, containment equipment, and facilities; wear lab coat and gloves; frequent handwashing is essential. Never eat in the lab. Caution should be used with sharps.

**Reporting:** Make note of the date and time of the incident and any relevant details. Inform principal investigator, fill out an [Injury](http://www.des.umd.edu/risk_comm/wcomp/form/wcomp.pdf) Report Form and also report incident to BSO. If recombinant, the incident must be reported to NIH Office of Biotechnology Activities.

# X. TRANSFER OR TRANSPORT OF BIOHAZARDOUS MATERIAL

*Describe your lab’s procedures for transporting biohazardous or potentially biohazardous materials here. This includes the transfer or transport of research materials (including animals) between campuses, between rooms/buildings, and the transfer of waste between rooms and/or buildings for autoclaving.*

*Explain in detail the materials that will be transported (e.g. – cell line, animal, fixed tissues, waste, etc.,)*

|  |  |  |  |
| --- | --- | --- | --- |
| **Material** | **BSL level** | **Building and Room#** | **Procedure (autoclave, microscope observation etc.)** |
|  |  |  |  |
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*PPE used:*

*Engineering Controls and equipment used for transportation:*

*Procedure:*

*Caution and Precautions:*

* Label the secondary container with emergency contact information
* PPE must never be worn in public areas
* Use a “PATH” label when transporting animal carcasses or pathology specimens
* IACUC procedures must be followed when transporting live animals
* Individuals transporting biohazards in a personal vehicle must complete Shipping Biological Materials Training and comply with DOT regulations
* Biological materials are not permitted on public transportation (e.g., campus shuttles)
* Contact RMS/ Biosafety immediately at 940-565-2109 (RMS) or 940-369-8513 (biosafety) **or** 940-369-7260 (**Biosafety Officer)** if biohazardous materials are spilled in public areas

# XI. SAFE HANDLING OF CRYOGENIC LIQUIDS

**Danger!  Vials immersed in liquid nitrogen may explode violently when removed!**  Wear face and eye protection!   Plastic vials (even Nunc vials with silicon O-rings) used for storing cells in liquid nitrogen are designed to be used in the liquid nitrogen vapor phase.  When immersed in the liquid phase, the liquid nitrogen frequently enters vials around the cold O-ring.  When vials are removed to room temperature, the liquid nitrogen in the vial immediately begins to boil.  Usually it escapes harmlessly past the seal.  Occasionally (about 1 out of 1000 vials), the seal is too tight, and the pressure causes a violent rupturing of the vial, sending shards of sharp plastic rocketing in unpredictable directions with sufficient energy to lacerate the face and cause severe eye injury.  When removing vials from liquid nitrogen, it is mandatory that you wear full face shields, pulled in to touch your chin so that shards can't fly under the shield.  If they fit, wear goggles underneath the face shield.

# IX. WORKING WITH ANIMALS (delete section if not applicable)

Animals must be housed, handled, and used in accordance with the federal Animal Welfare Act (P.L. 89-544, *et seq*) and the NIH *Guide for the Care and Use of Laboratory Animals*. All research involving animals must be done under a protocol approved by the University of North Texas IACUC and the IBC. The Director of Laboratory Animal Resources is responsible for assuring the safety and wellbeing of the research animals. Refer to the BMBL for biosafety requirements (<https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf>).

General Safety procedures for working with animals at ABSL-1 or 2 containments

* Access to the animal facilities is restricted to personnel who have been advised of the potential hazard & who have a need to enter the room for program or service purposes.
* Staff will be advised of increased risks for persons who are immunocompromised, pregnant, or for whom infection might be unusually hazardous
* Personnel will wear gloves when handling animals or animal bedding/products
* Personnel must wash their hands after handling cultures &/or animals, and before leaving the animal facility.
* Eating, drinking, handling contact lenses & applying cosmetics are not permitted in the animal rooms.
* Storing of food for human use is not permitted in animal rooms.
* Doors to animal rooms within the buildings are kept closed when animals are present. The building access doors are kept locked.
* Work surfaces are decontaminated after use or a spill of a viable material.
* An insect & rodent control program is in effect.
* Bedding & waste materials from animal cages are removed in such a manner as to minimize the creation of aerosols & BSL-2 waste is disposed of by autoclaving.
* Cages are decontaminated & washed after use.
* All waste & animal carcasses from the animal rooms are double bagged before removal from the building for incineration.
* Sharps shall be handled properly according to the relevant section of this manual, the BMBL, and the UNT Biosafety Manual.
* Broken glassware is not to be handled by hand but should be removed by mechanical means such as a broom & dustpan, tongs or forceps.
* Spills should be reported to the immediate supervisor, Vivarium Manager and contact Biosafety Officer, Dr. Veena Naik at veena.naik@unt.edu. And fill out a [RMS Incident Report](https://qafederation.ngwebsolutions.com/sp/startSSO.ping?PartnerIdpId=https://sso.unt.edu/idp/shibboleth&TargetResource=https%3a%2f%2fdynamicforms.ngwebsolutions.com%2fSubmit%2fStart%2faff08a6b-5197-4d92-ba2a-420290b3955b)
* All personnel entering animal rooms shall wear appropriate protective equipment.

# IX. WORKING WITH HUMAN SUBJECT (delete if not applicable)

* All Human Subject Research must have IRB and IBC approval in place if it involves collection of Biological Materials (i.e., cells, tissues, organs, blood or other bodily fluids)
* Access to the Lab spaces/clinic facilities is restricted to personnel who have been advised of the potential hazard & who have a need to enter the room for program or service purposes.
* Personnel will use proper PPE when handling human materials
* Personnel must wash their hands after interacting with in-person human subjects
* Eating, drinking, handling contact lenses & applying cosmetics are not permitted in the clinic or labs where human subjects or human derived research materials are managed or handled.
* Storing of food for human use is not permitted in labs/clinic where human subjects visit.
* Complete the shipping of biological material training before transporting or shipping biological material for clinical samples.

# X. LAB-SPECIFIC TRAINING RUBRIC and DOCUMENTATION

Insert methods for training lab staff in managing laboratory-specific hazards and training documentation. NOTE: Lab-**specific** training is REQUIRED and must be DOCUMENTED.

Provide information here –

Use the table below to record the details of the student who have competed this training -

**Laboratory Specific Training Records**

Lab specific training is required before the start of an experiment and at least annually thereafter. Attach this list with your IBC protocol submissions (New protocol, amendments or Annual Renewals).

Please use this form to document annual lab specific training. (Copy as needed)

|  |
| --- |
| **Principal Investigator:** |
| **Trainer:** |

**Topics covered during training include:**

*(Example topics can include biosafety manual review, emergency procedures, spill cleanup, exposure control plan, etc.). Develop and SOP or plan for the training you are providing and document that plan along with this record. Lab members should read the plan, understand those plan and hazards associated with it before signing the document. List such plans/SOP training below:*

1.
2.
3.
4.
5.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **UID** | **Date** | **Signature** |
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# Appendix 3. Principal Investigator’s Protocol Registration Record:

(Attach PDF copies of research forms submitted to RMS/BSO/IBC and approved by RMS/BSO/IBC. All work with biohazardous agents, r/s DNAs, human or non-human primate unfixed tissues, cells or blood, biological toxins, or select agents or toxins must be registered before materials are obtained and the work begins.)

# XI: SOPs

Attach safety and procedural SOPs. Per BMBL, manual must contain “the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed,” and contain protocols. BSO can provide template SOPs for safety procedures (handwashing, decon, etc.).

# XII: Risk Assessment

Please provide a protocol-specific risk assessment, you may need to provide more than one depending on the work being conducted. Risk assessments are described in the BMBL (<https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf>), and also on the UNT biosafety website (<https://riskmanagement.unt.edu/sites/default/files/01_how_to_complete_a_bio_risk_assessment.docx>).

|  |  |  |
| --- | --- | --- |
| What are the consequences of this incident occurring? Consider what could reasonably happen. Look at the descriptions and choose the most suitable consequence. | What is the likelihood of the consequence identified in step 1 happening? Consider this *without* new or interim controls in place. Look at the descriptions and choose the most suitable probability. | 1. Take severity rating and select the correct column
2. Take probability rating and select the correct line
3. The risk score is where the two rating cross on the matrix below. Add risk to chart.

**H= High M = Medium, L = Low** |
| **Severity** | **Probability** | **Risk Guide:** |
| **Consequence** | **Description** |  | **Description** |

|  |  |
| --- | --- |
|  | **Severity** |
| **Neg** | **Min** | **Ser** | **Crit** | **Cat** |
| **Probability** | **A** | **L** | **M** | **H** | **H** | **H** |
| **B** | **L** | **M** | **H** | **H** | **H** |
| **C** | **L** | **M** | **M** | **H** | **H** |
| **D** | **L** | **L** | **M** | **M** | **H** |
| **E** | **L** | **L** | **L** | **M** | **M** |

 |
| * **Catastrophic**
 | Death and extensive injuries | **A** | Frequent, >50% |
| * **Critical**
 | Life threatening | **B** | Probable 11%-50% |
| * **Serious**
 | Potential illness/impairment | **C** | Occasional, between 1%and 10% |
| * **Minor**
 | Material cost, first aid | **D** | Remote chance,<1% |
| * **Negligible**
 | Minor cost, no potential for illness | **E** | Improbable, once in the life of the measuring system, statistically insig. |  |

**STEP 1: IDENTIFY POTENTIAL AND EXISTING HAZARDS**

Select applicable hazards and assess their individual risk as, high, medium, or low by using the risk assessment matrix provided above. Space has been provided to list additional Hazards.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **r/sDNA Hazards** | **Risk**  | **Agent Hazards** | **Risk** | **Other Hazards** | **Risk** |
|[ ]  **Formation** - the creation of a genetically-altered organism through deliberate or accidental means. | Choose Risk |[ ]  **Pathogenicity**, virulence, and strain infectivity / communicability | Choose Risk |[ ]  **Host range-** Zoonosis: can the pathogen infect both animals and humans?  | Choose Risk  |
|[ ]  **Release** the deliberate release or accidental escape of some of these organisms in the workplace and/or into the environment | Choose Risk |[ ]  **Mode/Route of transmission** (mode of laboratory transmission may differ from natural transmission) | Choose Risk |[ ]  **Host factors—**can biohazard cause disease in healthy adult? What populations are at greater risk  | Choose Risk |
|[ ]  **Proliferation** - the subsequent multiplication, genetic reconstruction, growth, transport, modification and die-off of these organisms in the environment, including possible transfer of genetic material to other organisms. | Choose Risk |[ ]  **Infectious dose** (the number of microorganisms required to initiate infection can vary greatly with the specific organism, patient, and route of exposure) or LD50 for toxic materials | Choose Risk |[ ]  **Epidemiology—**is the biohazard endemic or foreign to the geographical research area? Is there a risk to the biohazard escaping the research facility and entering the environment? | Choose Risk Level. |
|[ ]  **Establishment** - the establishment of these organisms within an ecosystem niche, including possible colonization of humans or other biota. | Choose Risk |[ ]  **The risk of the formation of replication competent viruses** when using recombinant viral vectors | Choose Risk |[ ]  **The facility** (e.g., BSL-2, open floor plan [more risk] versus separate areas or rooms for specific activities [less risk], sufficient space versus crowded space, workflow, equipment present) | Choose Risk Level. |
|[ ]  **Effect** - the subsequent occurrence of human or ecological effects due to interaction of the organism with some host or environmental factor. | Choose Risk |[ ]  **Form** (stage) of the agent (e.g., presence or absence of cell wall, spore versus vegetation, conidia versus hyphae for mycotic agents) | Choose Risk  |[ ]  **The equipment** (e.g., uncertified BSCs, cracked centrifuge tubes, improperly maintained autoclaves, overfilled sharps containers, Bunsen burners) | Choose Risk |
|[ ]  **Gene Drive—**genetic engineering technology that propagates a particular suite of genes throughout a population by altering the probability that a specific allele will be transmitted to offspring from the natural 50% probability | Choose Risk |[ ]  **Invasiveness** of agent (ability to produce certain enzymes) | Choose Risk |[ ]  **Potential for generating aerosols and droplets** (Manipulating needles, syringes and sharps, Manipulating inoculation needles, loops, and pipettes, centrifugation, pouring, decanting, shaking) | Choose Risk |
|[ ]  **Genetic modifications** that alter the risk, such as expression of oncogenes or siRNAs to knockdown tumor suppressors | Choose Risk . |[ ]  **Stability** of biohazard | Choose Risk |[ ]  **Use of animals** | Choose Risk  |
|[ ]  **OTHER:\_\_\_\_\_\_\_\_\_\_\_\_****\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** | Choose Risk |[ ]  **OTHER:\_\_\_\_\_\_\_\_\_\_\_\_****\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** | Choose Risk  |[ ]  **OTHER: \_\_\_\_\_\_\_\_\_\_****\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** | Choose Risk  |

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| **Physical hazards** | **Risk**  | **Animal Hazards** | **Risk** | **Other Hazards** | **Risk** |
|[ ]  **Hot environment** (High UV, heat stress, dehydration | Choose Risk |[ ]  **Project animals** (bites, kicks, biological fluids, zoonotic diseases) | Choose Risk |[ ]  **Infectious agents:**Click or tap here to enter text. | Choose Risk  |
|[ ]  **Cold environment** (frost bite, Hypothermia, cold water) | Choose Risk |[ ]  **Bites and stings** (ticks, leeches, spiders, bees) | Choose Risk |[ ]  **Allergens** (pollen, poison ivy, wild parsnips) | Choose Risk |
|[ ]  **Electrical hazards**Click or tap here to enter text. | Choose Risk |[ ]  **Restraint equipment** Click or tap here to enter text. | Choose Risk |[ ]  **Participant injury/illness** | Choose Risk Level. |
|[ ]  **Hazardous equipment** (hammers, drills, etc.)Click or tap here to enter text. | Choose Risk |[ ]  **Large animal handling**Click or tap here to enter text. | Choose Risk |[ ]  **Working Alone** | Choose Risk Level. |
|[ ]  **Manual Work** (Lifting, pushing, pulling, digging) | Choose Risk |[ ]  **Vector-borne diseases** (West Nile virus, Lyme disease)Click or tap here to enter text. | Choose Risk  |[ ]  **Transportation accident/failure** | Choose Risk |
|[ ]  **Ergonomic Hazards**(repetitive motion) | Choose Risk |[ ]  **Project activities** (boating, swimming, climbing, all- terrain vehicles) | Choose Risk |[ ]  **Violent persons** | Choose Risk |
|[ ]  **Fatigue** (driving long hours) | Choose Risk . |[ ]  **Wildlife** (venomous snakes, scorpions, animal bites, Zoonotic diseases) | Choose Risk |[ ]  **Use or Storage of Hazardous Chemicals** (disinfectants, anaesthetics, medications) \*\*submit list to RMS | Choose Risk  |
|[ ]  **OTHER:**Click or tap here to enter text. | Choose Risk |[ ]  **OTHER:**Click or tap here to enter text. | Choose Risk  |[ ]  **OTHER:**Click or tap here to enter text. | Choose Risk  |

**STEP 2: RISK MITIGATION PLAN**

For hazards identified in Step 1, please list appropriate controls to eliminate or lessen the risk to project personnel. For hazards ranked H and M, mitigation must be in place and approved by RMS. Please be sure to include as many of the mitigation controls that you will be using as possible. This plan will be returned to you if it is incomplete or inadequate (i.e., if no PPE is included in your plan).

|  |  |  |
| --- | --- | --- |
| **Priority**  | **Control**  | **Example**  |
| 1.  | Eliminate  | Removing the hazard.  |
| 2.  | Substitute  | Replacing a hazardous process with a less hazardous one.  |
| 3.  | Isolation  | Isolating the hazard from the person at risk.  |
| 4.  | Engineering  | Redesign a process or piece of equipment to make it less hazardous.  |
| 5.  | Administrative  | Adopting safe work practices and providing appropriate training and instruction.  |
| 6. | PPE | Utilizing Personal Protective Equipment (PPE) to protect personnel |

|  |  |  |
| --- | --- | --- |
| Hazard | Problem | Controls |
| EXAMPLE: Working in/near Water  | Drowning  | Provide appropriate safety equipment, work in pairs, report back to PI/Supervisor when task is completed  |
| Click or tap here to enter text. | Click or tap here to enter text. | Click or tap here to enter text. |
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**Step 3: OVERALL RISK ASSESSMENT**

Taking into account the hazards identified in Step 1 and the likelihood and consequences of the hazards, assess the overall risk of the activity.

[ ] Low Risk

[ ] Medium Risk

[ ] High/Extreme Risk

Provide copies of risk assessment to all research staff. All participants must have the minimal level of skill, experience, training, and physical fitness to safely perform the activities. **All training must be documented.**

*This Risk Assessment is completed based on information provided on the referenced protocol. The Assessment does not identify each and every risk associated with this protocol.  The Principal Investigator (PI) has primary responsibility for overall health and safety for this protocol. If any changes effecting safety and health are made to this protocol, the PI is to contact the IBC and UNT Risk Management Services.*

**SIGNATURE and ACKNOWLEDGEMENT PAGE**

**Authorization** [*This section is based upon your specific laboratory setup*]

All members of the [*Principal Investigator’s*] Lab who have signed the list below are approved for entry into Room [*XXXX*] while work with BSL [*1 or 2*] agents is in progress. Anyone (including any workers not in the P.I.’s Lab) who uses the Room

[*XXXX*] lab must sign the disclaimer below.

# Disclaimer

We, the undersigned, understand the risks associated with the agents and activities with the work in [insert labs]. Further, we have read and understood this manual and agree to attend any Laboratory Safety training conducted by the [*Principal Investigator’s*] prior to performing work in Room [*XXXX*] .\*\***NOTE**\*\*PI or their designee **is required** to provide laboratory-specific training on safety (and procedures if appropriate) at least annually and document!

|  |  |  |
| --- | --- | --- |
| Name | Signature | Date |
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# Appendix 1. For Reference: BMBL BSL-1 and BSL-2 Laboratory Criteria

***Biosafety in Microbiological and Biomedical Laboratories (BMBL)***

**6th Edition, 2020,** [**https://www.cdc.gov/labs/BMBL.html**](https://www.cdc.gov/labs/BMBL.html)

**Centers for Disease Control and Prevention and National Institutes of Health**

**Biosafety Level 1**

Biosafety Level 1 (BSL-1) is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans and that present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open benchtops using standard microbiological practices. Special containment equipment or facility design is not generally required but may be used as determined by appropriate risk assessment. Laboratory personnel receive specific training in the procedures conducted in the laboratory and are supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility specifications are recommended for BSL-1.

* 1. A. **Standard Microbiological Practices**
1. The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.
2. The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained.

Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.

1. Personal health status may affect an individual’s susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance. See Section VII.
2. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.
	1. The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontami­nation methods, and the work performed.
	2. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
3. A sign is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory’s Biosafety Level, the supervisor’s or other responsible personnel’s name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.
4. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
5. Gloves are worn to protect hands from exposure to hazardous materials.
	1. Glove selection is based on an appropriate risk assessment.
	2. Gloves are not worn outside the laboratory.
	3. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
	4. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated laboratory waste.
6. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
7. Persons wash their hands after working with potentially hazardous materials and before leaving the laboratory.
8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas. Food is stored outside the laboratory area.
9. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
10. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:
	1. Plasticware is substituted for glassware whenever possible.
	2. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
		1. Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
		2. Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
		3. If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).
		4. Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.
	3. Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
	4. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
11. Perform all procedures to minimize the creation of splashes and/or aerosols.
12. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.
13. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
	1. a. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
	2. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
14. An effective integrated pest management program is implemented. See Appendix G.
15. Animals and plants not associated with the work being performed are not permitted in the laboratory.

**B. Special Practices** None required.

**C. Safety Equipment (Primary Barriers and Personal Protective Equipment)**

1. Special containment devices or equipment, such as biosafety cabinets (BSCs), are not generally required.
2. Protective laboratory coats, gowns, or uniforms are worn to prevent contamination of personal clothing.
3. Protective eyewear is worn by personnel when conducting procedures that have the potential to create splashes and sprays of microorganisms or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
4. In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

**D. Laboratory Facilities (Secondary Barriers)**

1. Laboratories have doors for access control.
2. Laboratories have a sink for handwashing.
3. An eyewash station is readily available in the laboratory.
4. The laboratory is designed so that it can be easily cleaned.
	1. Carpets and rugs in laboratories are not appropriate.
	2. Spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Laboratory furniture can support anticipated loads and uses.
	1. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
	2. b. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appro­priate disinfectant.
6. Laboratory windows that open to the exterior are fitted with screens.
7. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

**Biosafety Level 2**

Biosafety Level 2 (BSL-2) builds upon BSL-1. BSL-2 is suitable for work with agents associated with human disease and pose moderate hazards to personnel and the environment. BSL-2 differs from BSL-1 primarily because: 1) laboratory personnel receive specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility specifications are recommended for BSL-2.

**A. Standard Microbiological Practices**

1. The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.
2. The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
3. .Personal health status may affect an individual’s susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance. See Section VII.
4. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.
	1. The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
	2. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
5. A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory’s Biosafety Level, the supervisor’s or other responsible personnel’s name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.
6. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.
7. Gloves are worn to protect hands from exposure to hazardous materials.
	1. Glove selection is based on an appropriate risk assessment.
	2. Gloves are not worn outside the laboratory.
	3. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
	4. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated laboratory waste.
8. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
9. Persons wash their hands after working with potentially hazardous materials and before leaving the laboratory.
10. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas. Food is stored outside the laboratory area.
11. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
12. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:
	1. Plasticware is substituted for glassware whenever possible.
	2. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.
		1. Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
		2. Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
		3. If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).
		4. Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.
	3. Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
	4. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
13. Perform all procedures to minimize the creation of splashes and/or aerosols.
14. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.
15. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
	1. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
	2. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
16. An effective integrated pest management program is implemented. See Appendix G.
17. Animals and plants not associated with the work being performed are not permitted in the laboratory.

**B. Special Practices**

1. Access to the laboratory is controlled when work is being conducted.
2. The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate proficiency in standard microbiological practices and techniques for working with agents requiring BSL-2 containment.
3. Laboratory personnel are provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
4. Properly maintained BSCs or other physical containment devices are used, when possible, whenever:
	1. Procedures with a potential for creating infectious aerosols or splashes are conducted. These include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
	2. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotors or centrifuge safety cups with loading and unloading of the rotors and centrifuge safety cups in the BSC or another containment device.
	3. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of appropriate personal protective equipment and administrative controls are used, based on a risk assessment.
5. Laboratory equipment is decontaminated routinely; after spills, splashes, or other potential contamination; and before repair, maintenance, or removal from the laboratory.
6. A method for decontaminating all laboratory waste is available (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
7. Incidents that may result in exposure to infectious materials are immediately evaluated per institutional policies. All such incidents are reported to the laboratory supervisor and any other personnel designated by the institution. Appropriate records are maintained.

**C. Safety Equipment (Primary Barriers and Personal Protective Equipment).**

1. Protective laboratory coats, gowns, or uniforms designated for laboratory use are worn while working with hazardous materials and removed before leaving for non-laboratory areas (e.g., cafeteria, library, and administrative offices). Protective clothing is disposed of appropriately or deposited for laundering by the institution. Laboratory clothing is not taken home.
2. Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use.
3. The risk assessment considers whether respiratory protection is needed for the work with hazardous materials. If needed, relevant staff are enrolled in a properly constituted respiratory protection program.
4. In circumstances where research animals are present in the laboratory, the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

**D. Laboratory Facilities (Secondary Barriers)**

1. Laboratory doors are self-closing and have locks in accordance with the institutional policies.
2. Laboratories have a sink for handwashing. It should be located near the exit door.
3. An eyewash station is readily available in the laboratory.
4. The laboratory is designed so that it can be easily cleaned.
	1. Carpets and rugs in laboratories are not appropriate.
	2. Spaces between benches, cabinets, and equipment are accessible for cleaning.
5. 5.Laboratory furniture can support anticipated loads and uses.
	1. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
	2. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
6. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they are fitted with screens.
7. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
8. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent. See Appendix A, Figure 11. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment.
9. There are no specific requirements for ventilation systems. However, the planning of new facilities considers mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness. See Appendix A.
	1. BSCs are installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs are located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
	2. BSCs can be connected to the laboratory exhaust system by either a canopy connection (Class IIA only) or directly exhausted to the outside through a hard connection (Class IIB, IIC, or III). Class IIA or IIC BSC exhaust can be safely recirculated back into the laboratory environment if no volatile toxic chemicals are used in the cabinet.
	3. BSCs are certified at least annually to ensure correct performance, or as specified in Appendix A, Part 7.

**Animal Biosafety Level 1**

Animal Biosafety Level 1 (ABSL-1) is suitable for animal work involving well-characterized agents that are not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to personnel and the environment.

Special containment equipment or facility design may be required as determined by risk assessment. See Section II for additional information on the Biological Risk Assessment.

Personnel receive specific training in animal facility procedures and are supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

The following standard practices, safety equipment, and facility specifications are recommended for ABSL-1.

**A. Standard Microbiological Practices**

1. The animal facility director establishes and enforces policies, procedures, and protocols for biosafety, biosecurity, and emergencies within the animal facility.
2. Access to the animal room is limited. Only those persons required for experimental, husbandry, or support purposes are authorized to enter the facility.
3. Each institution ensures that worker safety and health concerns are addressed as part of the animal protocol review process. Consideration is given to specific biohazards unique to the animal species and protocol in use. Prior to beginning a study, animal protocols are reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) as well as the Institutional Biosafety Committee (IBC), as appropriate.
4. The supervisor ensures that animal care, facility, and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization). Personnel receive annual updates and additional training when equipment, procedures, or policies change. Records are maintained for all hazard evaluations, training sessions, and staff attendance. All persons, including facility equipment personnel, service workers, and visitors, are advised of the potential hazards (e.g., naturally acquired or research pathogens, allergens); are instructed on the appropriate safeguards; and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.
5. Personal health status may affect an individual’s susceptibility to infection or ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance. See Section VII. Facility supervisors ensure that medical staff are informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care, and manipulations.
6. Appropriate occupational medical services are in place, as determined by risk assessment.
7. An animal allergy prevention program is part of the medical surveillance.
8. Personnel using respirators for animal allergy prevention are enrolled in an appropriately constituted respiratory protection program.
9. A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated, as necessary.
10. The safety manual contains sufficient information to describe the biosafety and containment procedures for the experimental animals, organisms, and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
11. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility malfunctions, escape of animals within the animal facility, and other potential emergencies. A plan for the disposition of animals during emergency situations is included. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.
12. A sign is posted at the entrance to the animal room when infectious agents are present. Posted information includes: the room’s Animal Biosafety Level, the supervisor’s or other responsible personnel’s name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the animal room. Agent information is posted in accordance with the institutional policy.
13. Long hair is restrained so that it cannot contact hands, animals, specimens, containers, or equipment.
14. Gloves are worn to protect hands from exposure to hazardous materials and when handling animals.
15. Glove selection is based on an appropriate risk assessment.8–12
16. Consider the need for bite and/or scratch-resistant gloves.
17. Gloves worn inside the animal facility are not worn outside the animal facility.
18. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
19. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated animal facility waste.
20. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.
21. Persons wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or manipulated.
22. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in animal areas.
23. Mouth pipetting is prohibited. Mechanical pipetting devices are used.
24. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements.13 Whenever practical, supervisors adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions are always taken with sharp items. These include:
25. Plasticware is substituted for glassware whenever possible.
26. Use of needles and syringes or other sharp instruments is limited in the animal facility and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are used whenever possible.
27. Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.
28. Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
29. If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, or the use of forceps to hold the cap when recapping a needle).
30. Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.
31. Non-disposable sharps (e.g., necropsy instruments such as forceps, pins, reusable scalpels) are placed in a hard-walled container for transport to a processing area for decontamination.
32. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.
33. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
34. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the animal facility.
35. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport:
36. Materials to be decontaminated outside of the immediate animal room are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label.
37. Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.
38. An effective integrated pest management program is required. See Appendix G.
39. Animals and plants not associated with the work being performed are not permitted in the areas where infectious materials and/or animals are housed or manipulated.

**B. Special Practices None required.**

**C. Safety Equipment (Primary Barriers and Personal Protective Equipment)**

1. Specialized devices or equipment for restraint or containment may be required as determined by appropriate risk assessment.
2. Laboratory coats, gowns, or uniforms are the minimum recommended to prevent contamination of personal clothing. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the animal facility.
3. Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield, or other splatter guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated facility waste or decontaminated after use.
4. Persons having contact with NHPs assess the risk of mucous membrane exposure and wear protective equipment (e.g., face shield, surgical mask, goggles), as appropriate.
5. Additional PPE is considered for persons working with large animals.

**D. Animal Facilities (Secondary Barriers)**

* 1. ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Consider placing animal areas away from exterior walls of buildings to minimize the impact from the outside environment temperatures.
1. External facility doors are self-closing and self-locking.
2. Access to the animal facility is restricted.
3. Doors to areas where infectious materials and/or animals are housed open inward, are self-closing, are kept closed when experimental animals are present, and never propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
	1. The animal facility has a sink for handwashing.
	2. Emergency eyewash and shower are readily available, easily accessible, and appropriately maintained.
	3. Sink traps are filled with water and/or appropriate disinfectant to prevent the migration of vermin and gases.
	4. If open floor drains are provided, the traps are filled with water and/ or appropriate disinfectant or sealed to prevent the migration of vermin and gases.
	5. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (e.g., walls, floors, ceilings) are water-resistant.
4. Floors are slip-resistant, impervious to liquids, and resistant to chemicals. Floors with drains are sloped toward drains to facilitate cleaning.
5. It is recommended that penetrations in floors, walls, and ceilings be sealed, including openings around ducts, doors, doorframes, outlets, and switch plates to facilitate pest control and proper cleaning.
6. Internal facility fixtures, such as light features, air ducts, and utility pipes, are designed and installed to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
7. External windows are not recommended; if present, they are resistant to breakage. Where possible, windows are sealed. If the animal facility has windows that open, they are fitted with fly screens.
8. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

4. Furniture can support anticipated loads and uses.

1. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
2. Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and decontaminated with an appropriate disinfectant and sealed to prevent harboring of insects/vermin.
3. Equipment and furnishings are carefully evaluated to minimize exposure of personnel to pinch points and sharp edges and corners.

5. Ventilation is provided in accordance with the Guide for the Care and Use of Laboratory Animals.3

* 1. Ventilation system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
	2. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washers have a final rinse temperature of at least 180°F. If manual

# Appendix 2: REFERENCES

[*UNT Biosafety Manua*](https://riskmanagement.unt.edu/sites/default/files/01_unt_biosafety_manual_temporary.docx)*l:*

*Biosafety in Microbiological and Biomedical Laboratories*, 6th edition

 <https://www.cdc.gov/labs/BMBL.html>

*Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*

 <http://oba.od.nih.gov/rdna/nih_guidelines_oba.html>

# Appendix 3: AGENT SUMMARY STATEMENT (OPTIONAL)

The BMBL contains summary statements for many bacteria, viruses, and zoonotics. If your agent is listed in the *BMBL*‘s Agent Summary Statements section, you may find it useful to paste it into the biosafety manual. Canada’s Pathogen Safety Data Sheets are also very useful, and have many more agents listed.

<https://www.cdc.gov/labs/BMBL.html>

<https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html>