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# Human Neurological Tissue Used in Teaching and Research Safety Program

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# Introduction

These **Human Neurological Tissue Guidelines** outline the recommended health and safety program for conducting research and teaching activities with human neurological tissue at the University of Regina (U of R). This Program is intended for activities that use un-screened human neurological specimens, which are *not* suspected of containing prions but have not been confirmed clear.

These **Human Neurological Tissue Guidelines** apply to all U of R Faculty, Staff, Students, Contractors, and Visitors engaged in these activities.

The U of R *Biosafety for Education, Research, and Community Health Program* and*Health and Safety Policy* (GOV-100-005) provides the guidance and authority to the **Human Neurological Tissue Guidelines** and forms part of the Health and Safety Management System.

# Definitions

**Biological material** is pathogenic and non-pathogenic microorganisms, proteins, and nucleic acids, as well as any biological matter that may contain microorganisms, proteins, nucleic acids, or part thereof. Examples include, but are not limited to, bacteria, viruses, fungi, prions, toxins, GMOs, RNA, DNA, tissues samples, diagnostic specimens, and live vaccines.

**Biological Safety Cabinet** is a primary containment device that provides protection for personnel, the environment, and the product (depending on BSC class), when working with biological material.

**Biosafety** are the containment principles, technologies, and practices that are implemented to prevent unintentional exposure to infectious material and toxins, or their accidental release.

**Biosafety Officer (BSO)** is the individual designated by the Vice-President (Administration) to oversee the University biosafety and biosecurity practices.

**Community Health** refers to health, safety, and wellness initiatives directed towards all University Students, Faculty, Staff, and Community (Public) Members regardless of relationship with the University. This definition includes activities related to living, working, and learning on campus.

**Containment** is the combination of physical design parameters and operational practices that protect personnel, the immediate work environment, and the community from exposure to biological material.

**Containment** **Level (CL)** is the minimum physical containment and operational practice requirements for handling infectious material or toxins safely in laboratory and animal work environments. There are four containment levels ranging from a basic laboratory (CL1) to the highest level of containment.

**Contamination** is the presence of infectious material or toxins on a surface (e.g. bench top, hands, and gloves) or within other materials (e.g. laboratory samples, cell culture).

**Decontamination** is the process removing and/or inactivating infectious materials or toxins; this may be accomplished through disinfection or sterilization.

**Disinfection** is a process that eliminates most forms of living microorganisms; disinfection is much less lethal to infectious material than sterilization.

**Disinfectant** is any chemical agent used dominantly on inanimate objects to destroy or inhibit the growth of living microorganisms.

**Hazard** is any activity, situation, or substance that can cause or has the potential to cause illness or injury.

**Health, Safety & Environment** is the unit within Human Resources, that is available to assist faculty, staff, students, and visitors in making the University a safe place to live, work, and learn

**Human/Primary/Diagnostic/Clinical Specimen** is defined as any bodily substance taken from a person for the purpose of analysis, such as blood, urine, stool, tissue, and fluid.

**Incident** is an event or occurrence involving infectious material, infected animals, or toxins, including a spill, exposure, release of infectious material or toxins, animal escape, personnel injury or illness, missing infectious material or toxins, unauthorized entry into the containment zone, power failure, fire, explosion, flood, or other crisis situations (e.g., earthquake, hurricane). Incidents include laboratory- acquired infections.

**Infectious Agent/Material/Organism** is biological material that is pathogenic in nature (i.e. contains human and/or animal pathogens) and poses a risk to human and/or animal health.

**Laboratory (Lab)** is an area within a facility or the facility itself where biological material is handled and/or stored for *in vitro* and/or *in vivo* work.

**Laboratory (Lab) Manager** is the person most responsible for the activities being conducted and/ or most responsible for the personnel conducting activities in the lab work area.

**Limited Access** is the access to a containment zone that is limited to authorized personnel and is achieved through a controlled access system or operational procedures (i.e., CL2 lab work areas).

**Medical Surveillance Program** is the program designed to prevent and detect personnel illness related to exposure to infectious material or toxins. The focus of the program is primarily preventative, but provides a response mechanism through which a potential infectious can be identified and treated before serious injury and disease occurs.

**Pathogen** is a microorganism, nucleic acid, or protein capable of causing disease in humans and/or animals. Examples are listed in Schedule 2-4 or Part 2 of Schedule 5 of the HPTA but these are not exhaustive lists. Examples of animal pathogens can be found by visiting the CFIA website.

**Pathogen Safety Data Sheets (PSDS)** are technical documents describing the hazardous properties of pathogens and recommendations for the safe handling of them. A PSDS may include information such as pathogencity, drug susceptibility, first aid treatment, PPE, and risk group classification.

**Personal Protective Equipment (PPE)** is equipment and/or clothing worn by personnel to provide a barrier from infectious material or toxins, thereby minimizing the risk of exposure. PPE may include, but is not limited to, lab coats, gowns, full-body suits, gloves, protective footwear, safety glasses, safety goggles, masks, and respirators.

**Prion** is a small proteinaceous infectious particles generally accepted to be responsible for causing TSE disease in human and animals.

**Principle Investigator (PI)** is the holder of an independent grant administered by a university and the lead researcher for the grant project, usually in the sciences, such as a laboratory study or a clinical trial. The phrase is also often used as a synonym for "head of the laboratory" or "research group leader." While the expression is common in the sciences, it is used widely for the person or persons who make final decisions and supervise funding and expenditures on a given research project.

**Public Health Agency of Canada** promotes and protects the health of Canadians through leadership, partnership, innovation and action in public health.

**Restricted access** is access to a containment zone that is restricted to authorized personnel using a controlled access system (e.g., electronic access card, access code).

**Risk** is the probability of an undesirable event occurring and the consequences of that event.

**Risk Assessment** is a thorough review of all the risks based on the probability, severity, and frequency with which we are exposed to the hazard/ event.

**Safe/ Standard Operating Procedures (SOPs)** are specific safe operating procedures developed by the Principle Investigator, Laboratory Instructor, or individual responsible for the purchase, use, collection, storage, maintenance, and disposal of a biological substance.

**Safety Data Sheets** are an important component of [product stewardship](https://en.wikipedia.org/wiki/Product_stewardship) and [safety and health](https://en.wikipedia.org/wiki/Occupational_safety_and_health). It is intended to provide personnel with procedures for handling or working with that substance in a safe manner, and includes information such as physical data ([melting point](https://en.wikipedia.org/wiki/Melting_point), [boiling point](https://en.wikipedia.org/wiki/Boiling_point), [flash point](https://en.wikipedia.org/wiki/Flash_point), etc.), [toxicity](https://en.wikipedia.org/wiki/Toxicity), [health effects](https://en.wikipedia.org/wiki/Health_effect), [first aid](https://en.wikipedia.org/wiki/First_aid), [reactivity](https://en.wikipedia.org/wiki/Reactivity_%28chemistry%29), storage, disposal, protective equipment, and spill-handling procedures. SDS formats can vary from source to source within a country depending on national requirements.

**Supervisor** means a person who is authorized by the University to oversee or direct the work of employees or students, including, but not limited to, Deans, Directors, Department and Unit Heads, Academic Staff Members, and Managers

**Transmissible Spongiform Encephalopathy (TSE)** is a fatal neurodegenerative disease affecting humans and/or animals which is generally accepted to be caused by prions.

**(Biological) Toxin** is a poisonous substance that is produced or derived from a microorganism and can lead to adverse health effects in humans and/or animals. Human toxins are listed in Schedule 1 or Part 1 of Schedule 5 in the HPTA.

**Waste** is any solid of liquid material generated by a facility for disposal.

# Hazard Identification & Risk Assessments

Human neurological specimens may contain pathogens, and this should be considered when assessing the risks associated with working with this material. Based on the risk associated with the pathogen suspected of being within the sample and the testing activity, the physical and/ or operational requirements for activities with diagnostic specimens may sometimes be lower than the requirements for handling pure cultures.

In situations where it is suspected that a sample contains a pathogen from a risk group higher than the containment level of the testing facility (i.e., prion), additional operational practices or shipment to a facility with an appropriate containment level may be required.

This Program is intended for activities that use un-screened human neurological specimens, which are **not suspected of containing prions** but have not been definitively confirmed clear. There are no known effective treatments or vaccines for prions (also known a Transmissible Spongiform Encephalopathies or TSEs).Therefore, it is necessary to handle the neurological tissue with extreme caution, both for the researcher protection and for environmental protection.

Contact health.safety@uregina.ca for a complete hazard identification and risk assessment.

Before you start a new experiment or protocol, ensure you are adequately prepared:

1. Check resources: Is there a lab safe operating procedure for the technique already? Does anyone in the lab have experience with this protocol already?
2. Check the Safety Data Sheet (SDS) for every chemical you will be using – look at things like hazard class and reactivity; storage requirements (i.e., temp, light sensitive, etc.) – where should this chemical be stored? If the chemical is very hazardous, can you substitute this chemical for a less hazardous one or is there a less hazardous protocol you could use?
3. Proper PPE (e.g., type of gloves, should the experiment be performed in the fume hood or bio safety cabinet, does it need a face shield?)
4. What are the products or by-products of any reactions in your protocol? Are they hazardous (consider same questions asked above)? Do they require special waste disposal?
5. Check your detailed plan over with the PI or a senior lab member.
6. Prepare proper waste containers before starting your protocol – can the biological material be autoclaved or are the chemicals non-compatible? Does the tissue have to be incinerated? Review the Pathogen Safety Data Sheet (PSDS; if available) for guidance.
7. What happens if there is a spill?

Once you have identified hazards and determined the level of risk, the accepted mechanisms to control a hazard are:



**Elimination (Substitution):** Is there a pathogen or process that poses less of a risk that the one selected that will provide the same result?

**Engineering Controls:** This includes the selection and use of primary containment devices (e.g. primary containment caging, biological safety equipment, closed vessel, HVAC systems, etc.) Another example includes handling materials in specialized Containment Level Labs that have increased physical infrastructure safety requirements (e.g. sealed benches).

**Administrative Controls:** These are the controls that can alter the way in which the tasks are done and can include procedures and practices. For example, detailed procedures and training regarding how infectious waste is transported to the autoclave.

**PPE:** The PPE selected and worn by individuals can reduce or minimize the potential exposure to infectious materials or toxins. This is the last and least reliable line of defense.

These strategies should be developed, implemented, and regularly assessed and updated. The following pages will identify mitigation controls for some of the higher-risk biological hazards known on campus. Please contact health.safety@uregina.ca for assistance and guidance.

# Ethics

Researchers intending to perform activities with human specimens must ensure that all required ethics approvals from the Research Ethics Board (REB) are in place prior to engaging in any activities. The REB is responsible for the review of all ethics applications involving human subjects. For more information on the human ethics approval process and requirements, please refer to the U of R REB website, <http://www.uregina.ca/research/REB/main.shtml>.

# Training Requirements

## Health and Safety Training

All personnel must complete the University of Regina:

* Chemical and Laboratory Training (includes WHMIS 2015/ GHS and Fire Extinguisher Training)
* BioMedical Specimen Training
* Biosafety Level 2 Training
* Autoclave Training

Records of certification will be kept on file. Training is valid for three years.

## **Lab Specific Training**

PIs will provide information and arrange for training at the time of an individual's initial assignment to the lab. Lab activities will be supervised until training requirements are fulfilled. Personnel will demonstrate knowledge of and proficiency in Safe Operating Procedures (SOPs) and this training will be documented using **Appendix A**.

Laboratory personnel shall exhibit a willingness to follow established laboratory safety guidelines and adhere to SOPs. All personnel will read and adhere to the Public Health Agency of Canada’s (PHAC’s) *Canadian* *Biosafety Standards*, 2015 and to this Program.All personnel willread, understand, and sign an ***Authorized User Waiver*** outlining general lab safety requirements and expectations for human neurological tissue activities (See **Appendix B**). Written safe operating procedures must be available for personnel reference.

In addition, PIs will arrange for refresher training annually if there are any changes in processes or procedures.

## Emergency Response

On an annual basis, PIs will provide refresher training on procedures and protocols related to emergency response in the laboratory.

# Administrative Procedures

New SOPs and protocols must be approved by the PIs before initiation. Current SOPs, training requirements, and protocols (biosecurity, medical surveillance, etc.) will be reviewed and/or revised by PIs / the designatedLab Manager every **12 months**.

## Inspections

Regular visual inspections of the containment zone will be conducted and corrective actions will be documented. Every individual in the lab will be given a task to complete on a regular basis. This task will be documented using **Appendix C**.

## Building and Equipment Maintenance

Building and equipment maintenance, repair, inspection, testing, or certification, including performance verification is conducted and documented (minimum annually). Every individual in the lab will be given a task to complete on a regular basis. These tasks will be documented using **Appendix C**.

* Eyewash stations must be flushed weekly
* Verification of the integrity of primary containment devices to be performed
* Decontamination technology and process to be validated

# Description of Laboratory

## Commissioning

### General

At the U of R, building space design is developed, reviewed, and completed according to the National Building Code of Canada, National Fire Code of Canada, and other applicable codes and standards. Lab space can only be assigned by Facilities Management.

Some biological containment labs at the U of R must meet additional engineering, operational, technical, and physical requirements set by the U of R, Public Health Agency of Canada, and Canadian Food Inspection Agency.

### Biological Laboratory Containment Classification

All human neurological tissue activities must be conducted in a Containment Level 2 Laboratory that has been commissioned by the UofR Biological Safety Officer according to the Public Health Agency of Canada *Canadian Biosafety Standards*, 2015. The locations of the sinks, eyewash, safety showers, and fire extinguisher must be near the lab exit.

### Biological Laboratory Decommissioning Procedures

**All PIs, LIs, Lab Managers, and Supervisors who terminate or relocate their CL2 and CL2+ Lab activities at the U of R must contact the BSO (**health.safety@uregina.ca**) for assistance before starting the decommissioning process.**

## Restricted Access

Only users listed in **Appendix A** are approved for entry into a CL2 Lab without the permission of the PI. All others must first be authorized by the PI, must sign the visitor sheet, and be accompanied by a trained individual. Visitors, maintenance staff, custodial staff and others, as deemed appropriate, must be provided with training and/or supervision commensurate with their anticipated activities in the containment area. Up-to-date campus-wide signage provides contact information for entry.

If entry into these areas is essential to maintain the building, the BSO is available to provide the necessary orientation for staff or contractors required to enter these restricted laboratories in emergencies.

## Dedicated Administrative and Personal Areas

Dedicated administrative areas are pre-determined and must be kept clean and free of all lab materials. If these areas are “dirtied” then a thorough (30 minute contact time) chemical disinfection with bleach is required (see Laboratory SOPs).

Personal belongings (i.e. iPods, lap tops, watches) are to be kept away from areas where biohazards are handled or stored. Personal clothing is always stored separately from dedicated PPE in office spaces/ personal areas (not the administrative areas).

## Traffic Flow

As much as the physical design of the lab allows for, the lab traffic flow will move from clean to dirty. “Dirty areas” for manipulation of biohazards will be separated from the normal lab areas and the “clean administrative areas” (see above) will be completely isolated from all lab activities.

# General Laboratory Safety Considerations

1. Your safety comes first - lab personnel must immediately notify the PI in case of an accident, injury, illness, or exposure associated with lab activities.  Exposures must be reported to the BSO immediately.
2. No eating, drinking, smoking, handling contact lenses, or applying cosmetics in the lab at any time. Long hair must be tied back. Dangly jewelry, lanyards, scarves, ties, etc. must be removed.
3. No animals or minors (persons under the age of 18) will be allowed to enter the lab at any time.
4. Students and Staff will notify the PI of any existing medical conditions that could render them immuno-compromised (e.g. through radiation therapy or chemotherapy, pregnancy, diabetes, etc.) immediately.
5. Food, medications, or cosmetics should not be brought into the lab for storage or later use.  Food is stored outside in areas designated specifically for that purpose.
6. No open-foot shoes or sandals are allowed in the laboratory. Long pants are required.
7. All skin cuts, abrasions, ulcers, areas of dermatitis, etc. should be covered with a bandage.
8. Contact of face or mucous membranes with contaminated or potentially contaminated items are prohibited.
9. Mouth pipetting is prohibited; mechanical pipetting devices are to be used at all times.
10. Experimentally infecting cells or other specimens derived from the person conducting the experiment is prohibited.
11. All procedures are to be performed carefully to minimize the creation of splashes or aerosols; use biological safety cabinets when possible.
12. Follow all manufacturers’ instructions and lab SOPs when using any of the lab equipment.
13. Floors and bench to be kept clean, free from materials that are in excess, not required, or that cannot be easily decontaminated.
14. Wash hands:
* after removing gloves, and
* before leaving the laboratory.

# Biosafety/ Exposure Control Considerations

## Universal Precautions

All individuals shall adhere to universal precaution strategies when working with unscreened human/ primary specimens. For information, please see: <http://www.ccohs.ca/oshanswers/prevention/universa.html>.

## Prion Hazards

There are no known effective treatments or vaccines for prion (also known as Transmissible Spongiform Encephalopathies or TSEs), therefore, it is necessary to handle neurological tissues that could contain prions with extreme caution, both for researcher protection and for environmental protection.

* The infectious agents that transmit prion diseases are resistant to inactivation by heat and chemicals.
* Prion diseases are transmissible by inoculation or ingestion of infected tissues or homogenates, and infectivity is present at high levels in brain or other central nervous system tissues, and at slightly lower levels in lymphoid tissues including spleen, lymph nodes, gut, bone marrow, and blood.
* One of the main precautions to be taken when working with prions is to avoid puncture of the skin. Therefore, sharps and glass should not be used unless it has been determined that there is no other alternative.
* Prions are characterized by resistance to conventional inactivation procedures including irradiation, boiling, dry heat, and chemicals (formalin, betapropiolactone, alcohols).
* The use of conventional autoclaves as the sole treatment has not resulted in complete inactivation of prions.
* Formalin-fixed and paraffin-embedded tissues, especially of the brain, remain infectious.

## Medical & Health Surveillance

Faculty, Staff, Students, Contractors, and Visitors should notify the PI of any existing medical conditions that could render them immuno-compromised (e.g. through radiation therapy or chemotherapy, pregnancy, diabetes, etc.) as this puts them at greater risk for infection. Duty/ project accommodations and modifications would be implemented, including material handling restrictions, additional protective equipment, etc.

It is the responsibility of the PI who has been granted authorization to perform unscreened human neurological tissue procedures to ensure that all personnel are offered recommended immunizations when performing activities (see **Table 1** below). Personnel may formally decline immunization. Copies of all immunization records must be maintained by the supervising researcher. Contact health.safety@uregina.ca for more information.

**Table 1**

| **Recommended immunizations for U of R personnel working with human/ primary specimens** |
| --- |
| **Vaccine** | **Recommendation(s)** |
| **Diphtheria Tetanus** | All Researchers or lab staff should be immune. Primary series if no previous immunization. Booster doses of Td vaccine every 10 years. *(Available as Td or Tdap or Tdap-IPV. Tdap is indicated if an adult pertussis dose is needed. Tdap-IPV is indicated if both pertussis and polio vaccinations are needed.)* |
| **Hepatitis A & B** | If no evidence of immunity. *(Post-immunization serologic testing within 1 to 6 months of completion of primary series.)* |
|  |  |
| **Measles** | If no evidence of immunity, regardless of age - 2 doses. |
|  |  |
| **Mumps** | If no evidence of immunity, regardless of age - 2 doses. |
| **Pertussis** | A single dose of Tdap vaccine if not previously received in adulthood.  |
| **Polio** | Primary series if no previous immunization – 3 doses. Unvaccinated Researchers and Lab staff at highest risk of exposure should be particularly targeted for primary immunization.A single lifetime booster dose for Researchers and Lab staff at highest risk of exposure. |
| **Rubella** | If no evidence of immunity – 1 dose. |
|  |  |
| **Varicella** | If no evidence of immunity - 2 doses. (*Self-reported history of varicella or herpes zoster is not reliable for a Researcher or Lab staff to be considered immune.)* |

For more information, please see PHAC’s *Canadian Immunization Guideline*, (7th Edition, 2006).

## Personal Protective Equipment

The minimum personal protective equipment (PPE) required when performing human neurological tissue manipulations are:

* Lab coat;
* Safety glasses or full face protection;
* Double disposal non-latex gloves (e.g. nitrile gloves);
* Pants; and
* Closed toe/heel shoes.

## Biological Safety Cabinet

All manipulations with human neurological tissues (screened and unscreened) should be done in a biological safety cabinet. See Section #15 for safe operating procedures.

# Emergency Response

All exposures and incidents must be immediately reported to the PI.

## Emergency Contact Information

**24 Hour Emergency (Fire, Police, Medical):** 911

**24 Hour Saskatchewan Health Hotline:** 811

**Campus Security:** 306-585-4999

**Dr. X 306-**

**Biosafety Officer (BSO):** 306-585-5198/ 306-527-4320

**Health, Safety & Environment, Human Resources:** 306-585-4776

## Medical Emergency

1. Phone 911 – Direct them to the scene of the occurrence.
2. Call Campus Security: 306-585-4999
3. Give First Aid, if you are qualified to do so, or get help from Campus Security.
4. Stay with victim.

## Needle Stick Poke, Puncture Wound, or Percutaneous Injury

1. Remove gloves and allow the wound to bleed.
2. Immediately wash the affected area for 15 minutes with soap and warm water.
3. Notify Supervisor (if available) to obtain assistance.
4. Seek medical assistance immediately (within 1-2 hours) from a health care professional. The cause of the wound and organisms involved should be reported.
5. Details of the incident must be documented using the *Incident Report Form* and forwarded to Health, Safety & Environment within 24 hours. Forms can be found online at [www.uregina.ca/hr/hse](http://www.uregina.ca/hr/hse) or by contacting health.safety@uregina.ca or 306-585-4776. Please include the following details:
6. What was the method of contact (e.g. needle stick, splash)?
7. How did the exposure occur?
8. What known biological agents or body fluids were you in contact with?
9. What action was taken in response to the exposure to remove the contamination (e.g. hand washing)?
10. What personal protective equipment was being used at the time of exposure?
11. What is your immune status (e.g. Tetanus, Hepatitis A or B Virus)?

## Eyes or Mucous Membrane Exposure (e.g. Splash)

1. Immediately flush the affected area for 15 minutes using an eyewash or shower. Hold eyelids open and remove contact lens, if safe to do so, to ensure adequate flushing.
2. Notify Supervisor (if available) to obtain assistance.
3. Seek medical assistance immediately (within 1-2 hours) from a health care professional. The organisms involved should be reported.
4. Details of the incident must be documented using the *Incident Report Form* and forwarded to Health, Safety & Environment within 24 hours. Forms can be found online at [www.uregina.ca/hr/hse](http://www.uregina.ca/hr/hse) or by contacting health.safety@uregina.ca or 306-585-4776. Please include details as listed above.

## Ingestion

1. Notify Supervisor (if available) to obtain assistance.
2. Seek medical assistance immediately (within 1-2 hours) from a health care professional.
3. Identification of the material ingested and circumstances of the incident should be reported.
4. Details of the incident must be documented using the *Incident Report Form* and forwarded to Health, Safety & Environment within 24 hours. Forms can be found online at [www.uregina.ca/hr/hse](http://www.uregina.ca/hr/hse) or by contacting health.safety@uregina.ca or 306-585-4776. Please include details as listed above.

## Post-Exposure Procedures

If a student or employee has been exposed to biologically hazardous substances at the U of R, the University will, with the consent of the student/employee, during the student/employee’s normal working hours, arrange for immediate medical evaluation, medical intervention, and confidential post-exposure counselling. If a student/employee cannot receive medical evaluation, medical intervention, or post-exposure counselling during the student/employee’s normal working hours, the U of R will credit the student/employee’s attendance for evaluation, intervention, or counselling as time at work and shall ensure that the student/employee does not lose any pay or other benefits.

## Fire Extinguisher

Fire Extinguisher is located at exit/ entry of main labs.

1. Fire extinguishers should be used only if the fire is small and confined to one small area!
2. To operate, pull the pin to release the handle.
3. Stand at a safe distance from the fire (as directed on the fire extinguisher).
4. Aim the nozzle at the base of the fire, squeeze the handle to discharge the agent, and sweep completely left and right until a few seconds after seeing no fire.

# Biological Material Spills

The most immediate concern following a spill of biologically hazardous materials or organisms is to contain the spill and treat any exposed persons. After this occurs, properly trained personnel can begin the clean up and decontamination process.

Every CL2 and CL2+ lab must have basic supplies to assist with biologically hazardous spill cleanup. The kit must contain:

* Personal protective equipment
* Forceps and sharps waste disposal container
* Concentrated disinfectant (effective against organism of use)
* Paper towels
* Autoclave/biohazard bags

The Hazardous Material Spill Response Team (contacted via Campus Security (4999)) can assist with biological material spill cleanup.

## Small Non-Hazardous Biological Spill

(Spills that you are comfortable cleaning up)

1. All persons should inform other personnel in the affected area not to enter.
2. Review the MSDS and PSDS, to determine the protective equipment, spill cleanup, and disposal protocols that are necessary for all chemicals and biological materials involved.
3. Wear gloves, lab coat, shoes, pants, face and eye protection, and other appropriate personal protective equipment.
4. Cover the spill with cloth or paper towels to contain it.
5. Spray or pour an appropriate disinfectant over the paper towels and the immediate surrounding area (according to the specific biological PSDS; generally, 10% bleach or 70% ethanol solutions are appropriate).
6. Start applying the disinfectant from the outside and move inwards.
7. After the appropriate amount of time (15 - 30 minutes), clear away any materials like broken glass using forceps or another mechanical device and place in a sharps container/biohazard container.
8. Clean and disinfect the spillage area using paper towels and other appropriate cleaning materials.
9. Place contaminated materials into a labelled, leak-proof, puncture-resistant waste disposal container and dispose of waste appropriately. Contact Health, Safety & Environment (health.safety@uregina.ca) for waste disposal assistance.
10. Complete an **Incident Report Form** and forward to Health, Safety & Environment within 24 hours. Forms can be found online [www.uregina.ca/hr/hse](http://www.uregina.ca/hr/hse) or by contacting health.safety@uregina.ca.

## Large Non-Hazardous Biological Spill

(Spills you are not comfortable cleaning up by yourself)

1. All persons should inform other personnel in the affected area not to enter.
2. Review the MSDS and PSDS, to determine the protective equipment, spill cleanup, and disposal protocols that are necessary for all chemicals and biological materials involved.
3. PI and UR Hazardous Material Spill Response Team (via Campus Security (306-585- 4999)) should be informed for cleanup assistance.

## Small Hazardous Biological Spill

(Spills you are comfortable cleaning up)

1. All persons should immediately leave the affected area and allow aerosols to settle (~30 minutes).
2. Signs should be posted indicating that entry into area is forbidden. Post a sign stating “DO NOT ENTER, BIOHAZARD SPILL. Contact (name and phone #) for information.”
3. Any exposed person should seek medical assistance immediately (within 1-2 hours) from a health care professional.
4. PI, Health, Safety & Environment (306-585-4776), and/or a “Spill Buddy” should be informed for cleanup assistance.
5. Wear nitrile gloves, lab coat, shoes, pants, face and eye protection, and other appropriate personal protective equipment.
6. Cover the spill with cloth or paper towels to contain it.
7. Spray or pour an appropriate disinfectant over the paper towels and the immediate surrounding area (according to the specific biological PSDS; generally, 10% bleach solutions are appropriate).
8. Start applying the disinfectant from the outside and move inwards.
9. After the appropriate amount of time (see PSDS), clear away any materials like broken glass using forceps or another mechanical device and place in a sharps container/biohazard container.
10. Clean and disinfect the spillage area using paper towels and other appropriate cleaning materials.
11. Place contaminated cleaning materials into a labelled, leak-proof, puncture-resistant waste disposal container and dispose of waste appropriately. Contact Health, Safety & Environment (health.safety@uregina.ca) for waste disposal assistance.
12. Complete an **Incident Report Form** and forward to Health, Safety & Environment within 24 hours. Forms can be found online [www.uregina.ca/hr/hse](http://www.uregina.ca/hr/hse) or by contacting health.safety@uregina.ca.

## Large Hazardous Biological Spill

(Spills you are not comfortable cleaning up)

1. All persons should immediately leave the affected area and allow aerosols to settle (~30 minutes).
2. Signs should be posted indicating that entry into area is forbidden; post a sign stating “DO NOT ENTER, BIOHAZARD SPILL. Contact (name and phone #) for information.”
3. Any exposed person should seek medical assistance immediately (within 1-2 hours) from a health care professional.
4. PI and UR Hazardous Material Spill Response Team (via Campus Security (306-585- 4999)) should be informed for cleanup assistance.

## Potentially Hazardous Aerosol Release

1. All persons should immediately leave the affected area and no one should enter the room for an appropriate amount of time (e.g. 30 minutes), to allow for aerosols to be carried away and heavier particles to settle. If the lab does not have a central air exhaust system, entry should be delayed (e.g. for 24 hours).
2. Signs should be posted indicating that entry is forbidden. Post a sign stating “DO NOT ENTER, BIOHAZARD SPILL. Contact (name and phone #) for information.”
3. Any exposed person should seek **medical assistance immediately** (within **1-2 hours**) from a health care professional.
4. PI and UR Hazardous Material Spill Response Team (contacted via Campus Security (306-585-4999)) should be informed for cleanup assistance.
5. After the appropriate amount of time (~30 minutes – 24 hours), spill cleanup will proceed.

###

## Biological Spill and Broken Containers

1. All persons should immediately leave the affected area.
2. Any exposed person should seek **medical assistance immediately** (within **1-2 hours**) from a health care professional.
3. Determine if you are comfortable cleaning up the spill or require some assistance. Follow the above directions.

**Additional Considerations:**

1. Broken containers contaminated with infectious substances and spilled infectious substances should be covered with a cloth or paper towels. Care must be taken to avoid splashing or generating aerosols during the clean up.
2. Glass fragments should be handled with forceps or another mechanical device and placed in a sharps container/biohazard container. NEVER with your hand.
3. If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant for 30 minutes.
4. If lab forms or other printed or written material are contaminated, the information should be copied onto another form and the original discarded into the contaminated-waste container.

## Breakage of Tubes/ Leaking Tubes in Centrifuges

1. If breakage occurs or is suspected while the machine is running, the motor should be switched off and machine left closed for about **30 minutes** to allow for material settling.
2. If breakage is discovered after the machine has stopped, immediately close the lid and leave closed for about **30 minutes**.
3. Put on nitrile gloves, laboratory coat, shoes, pants, and eye/face protection. Strong, thick rubber gloves worn under the disposable nitrile gloves should be considered.
4. Before attempting to deal with the leak, prepare a pan of disinfectant solution large enough to immerse the entire rotor in (iodine-based solutions are recommended over bleach because of corrosion).
5. Get sharps waste container and forceps/tongs. Forceps or cotton held in forceps should be used to retrieve all glass debris.
6. If the rotor is sealed, and removable, remove rotor from the centrifuge using paper towels to catch drips.
7. Place leaking rotor in secondary containment and only open in the biological safety cabinet.
8. Carefully remove the lid from the rotor. All broken tubes, glass fragments, buckets, trunnions, and the rotor should be placed in the disinfectant.
9. Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered.
10. Wipe outside of rotor with disinfectant, and leave rotor in the cabinet, out of the way. All instruments and rotor pieces involved in the incident should be chemically decontaminated.
11. After proper decontamination, instruments and rotor pieces may be washed with a mild disinfectant or soap according to the manufacturer’s instructions.
12. As an added measure of caution, the inside of the centrifuge (chamber) must be wiped out with a non-corrosive disinfectant twice, washed with water, and dried.
13. If a tube has broken in a centrifuge that does not use a containment type rotor, DO NOT open the centrifuge. Turn off the power and allow sufficient time for aerosols that have been created to settle (~30 min.). Put on PPE and respiratory protection (**Must be Fit-Tested by HSE**) before opening the chamber. Decontaminate the inside of the chamber with a noncorrosive disinfectant (70% ethanol or Wescodyne) by thoroughly soaking the interior. A spray bottle or lab squeeze bottle is sufficient. Large amounts of liquid generated during decontamination may be removed by a disposable pipette attached to suction device attached to a disinfectant trap. Any paper waste generated during clean-up should be bagged and autoclaved.

**Note:** It is recommended that a sealed rotor or bucket be used when centrifuging infectious materials. If none is available, placing a smaller tube inside a larger sealable tube can provide some protection against aerosol creation in the event of breakage.

# Entry/ Exit Procedures (PPE)

## Authorized Users -Entry

1. Place all personnel items (bags, coats, laptop, etc.) in “administrative/clean areas” only (see *Administrative Procedures*).
2. Put on lab coat, nitrile gloves, pants, and closed-foot shoes.
	1. When handling biological materials, protective clothing, including gloves and a long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) should be worn so that hands and arms are completely covered to prevent contamination of cultures, skin, and street clothing.
	2. Eye protection/ face protection should be worn when handling infectious organisms and there is a likelihood of splash or flying object.
	3. These requirements also apply to anyone working in the area while someone else is working with biological materials.
3. Gather all materials for the experiment.

## Authorized Users – Exit

1. Before exiting the lab, be sure that the hood and work area are clean, everything is labeled, all contaminated waste materials are disposed of properly, and stocks have been returned to the proper storage area.
2. Remove PPE (In order: lab coat, eye wear, gloves).

**Steps for Removing Laboratory Coat:**

|  |  |  |  |
| --- | --- | --- | --- |
| 20140820_133057 | 20140820_133138 | 20140820_133148 | 20140820_133158 |
| 1. With gloves still on, unbutton coat.
 | 1. Pull off one arm, keep coat away from body.
 | 1. Pull off second arm, keeping coat away from body.
 | 1. Once coat is off, hold away from body and slowly roll coat.
 |

**Steps for Removing Eye Wear:**

|  |  |
| --- | --- |
| 20140820_133250 | 20140820_133258 |
| 1. Without touching face, grasp goggles with one gloved-hand.
 | 1. Pull goggles upward away and off of head.
 |

**Steps for Removing Gloves:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 20140820_133338 | 20140820_133345 | 20140820_133357 | 20140820_133401 | 20140820_133409 |
| 1. Grasp one glove on the inside of wrist at ½ inch below band of dirty side of glove without touching the skin.
 | 1. Pull down glove, turning it inside out, and pull hand. Hold the glove with the still-gloved hand.
 | 1. Insert fingers of ungloved hand under the cuff of the glove on the other hand (on inside of cuff).
 | 1. Pull down glove until it is inside out, drawing it over the first glove.
 | 1. Dispose of gloves in the garbage receptacle.
 |

1. Wash your hands very well.

**Hand Washing Steps:**

1. Remove all rings and wet your hands with warm running water.
2. Use soap and produce lather, rubbing your hands for 20 seconds.
3. Scrub all surfaces of hands including backs of hands, wrists, between fingers, and under fingernails. For best results use a nail brush.
4. Rinse hands in clean, warm running water for at least 10 seconds.
5. Dry hands with a clean towel.

\*Diagram taken from Canadian Center for Occupational Health and Safety

## Visitors – Entry

1. Determine required activities in LB406. (What PPE is required for these activities?)
2. Schedule time that no hazardous work is being conducted.
3. Clear immediate and surrounding areas of equipment, tools, chemicals, bottles, etc.
4. Disinfect (15 minute contact time minimum) immediate and surrounding floor, bench, cupboard, equipment, etc. with fresh 10% bleach solution.
5. If work is scheduled for a time that no one can be present to orientate/supervise, arrange time for the BSO (306.585.5198/306.527.4320/ health.safety@uregina.ca) to assist.
6. Once equipment and areas have been cleared and disinfected, place “Decontaminated” sign on equipment/area to indicate the decontamination procedure has been complete and Visitors can conduct activities.
7. Training and/or Supervision of Visitors needs to be considered based on experience and activity.
8. Remind Visitors to sign Sign-In Log Book. This is located on door.

## Visitors - Exit

1. If PPE was required, instruct Visitors how to remove PPE properly and safely (see above).
2. Remind Visitors to wash hands and to sign-out once duty is complete.

# Transfer and Transportation

## Transport and Removal of Waste, Samples, etc. from Laboratory

Transport of human neurological tissue to another building or lab/ autoclave within the same building should be done in a covered and sealed container. Use a secondary container and label it with the contents and a contact person/phone number.

### Liquid Waste

1. Wear gloves, laboratory coats, shoes, and other appropriate personal protective equipment (face and eye protection).
2. Samples or waste must be stored in a tightly closed tube, jug, pail, etc. If it is unrealistic to cap every tube, then samples must be stored in a tightly closed plastic container (*e.g.* Rubbermaid bin, Ziploc container, etc.) as the primary container.
3. Samples in primary container (tubes, jugs, containers) must be transported in a secondary container (*e.g.* autoclave bin, Rubbermaid bin, Ziploc container, etc.). Especially if materials are being transported through public hallways.
4. Wipe down storage and transport containers with 10% bleach or other disinfectant, if containers are contaminated or suspected to be contaminated.
5. Either remove PPE, use a buddy, or one ungloved hand to open door and press elevator buttons.
6. Transport liquid waste using a cart.

### Solid Waste

1. Wear gloves, laboratory coats, shoes, and other appropriate personal protective equipment (face and eye protection).
2. Samples or waste must be stored in an autoclave/ biohazard bag placed inside a tightly closed plastic container (e.g. Rubbermaid bin, Ziploc container, etc.).
3. Sample/ waste in primary container must be transported in a secondary container (e.g. autoclave bin, Rubbermaid bin, Ziploc container, etc.).
4. Wipe down storage and transport containers with 10% bleach, if containers are contaminated or suspected to be contaminated.
5. Either remove PPE, use a buddy, or one ungloved hand to open door and press elevator buttons.
6. Transport solid waste using a cart.

## Removal of Equipment from Laboratory

All pieces of equipment and wares must be properly decontaminated using a 10% bleach or 70% ethanol solution before removal from containment laboratory areas. As bleach may corrode metal, a 70% ethanol solution can be substituted for metal equipment decontamination.

1. Wear gloves, laboratory coats, shoes, and other appropriate personal protective equipment (face and eye protection).
2. Wipe all surfaces thoroughly with a cloth moistened with a disinfectant solution. Refer to manufacturer’s instructions for required contact time to disinfect the surface (minimum of 30 minutes).
3. For carpet or upholstered surfaces, a low level disinfectant may be used. Add the disinfectant to the regular carpet shampoo, according to manufacturer’s instructions.
4. Once equipment has been disinfected, place “Decontaminated” sign on equipment to indicate the decontamination procedure has been complete and equipment can be removed.

# Decontamination and Waste Procedures

See <http://bit.ly/bio-waste> for more waste disposal information.

## Protective and Personal Clothing Decontamination

All contaminated personal clothing items and non-disposable lab coats should be properly decontaminated to reduce risk of transmission and exposure. It is recommended that decontamination be performed every 6 months, but this will vary with the type and intensity of research activity.

1. Do not walk into public areas with contaminated clothing.
2. Promptly don the appropriate PPE for removing contaminated clothing (i.e. gloves).
3. If soiled clothing cleaning and disinfecting procedures cannot be completed in the room that the clothing was soiled, the items must be removed and transported in strong biohazard/plastic bags.
4. Soiled clothing should be handled as little as possible and with minimum agitation.
5. Hold the soiled clothing away from your unsoiled clothing.
6. Store the soiled clothing sealed in a thick biohazard/plastic bag.

## Chemical Disinfectant

* If possible, refer to pathogen safety data sheets for contact time and effective disinfectant.
* Materials should be immersed in a solution of bleach (household bleach diluted 1 part with 9 parts water, or 10% (v/v)) for **minimum of 30 minutes** before any further handling.
* Immersion in 70% ethanol for **30 minutes** may be an acceptable means of decontamination for items that are incompatible with bleach (metal and rubber objects).
* Disinfectant will be used to wipe down the benches, equipment (centrifuges, incubators), wares, and tools **daily** **and/ or after use**.
* Volatile or organic solvents such as fixatives that are, by their nature, toxic to biological materials need not be chemically decontaminated or autoclaved.
* Do not leave blood on dissection equipment and trays – thoroughly wash.

### Liquid Waste

1. Always wear nitrile gloves, lab coat, pants, closed-foot shoes, and safety glasses when handling chemical disinfectant.
2. Prepare bleach solution (household bleach diluted 1 part with 9 parts water, or 10% (v/v))
3. Add bleach to waste container.
4. Also rinse the vessel with bleach provided in the wash bottle in the cabinet.
5. Mix well and allow a minimum of **30 minute contact time** before being poured into the drain. Rinse with copious amounts of cold tap water. Liquid waste that is not compatible with bleach should be autoclaved for at least 30 minutes using slow exhaust before disposal; if items can be autoclaved (see below).

## Decontamination of Equipment in Laboratory

All pieces of equipment must be properly decontaminated using a 10% bleach or 70% ethanol dilution before maintenance of equipment and after use. As bleach may corrode metal, 70% ethanol can be substituted for metal equipment decontamination.

1. Always wear nitrile gloves, lab coat, pants, closed-foot shoes, and safety glasses when handling chemical disinfectant.
2. Wipe all surfaces thoroughly with a cloth moistened with a disinfectant solution. Refer to manufacturer’s instructions for required contact time to disinfect the surface (minimum of 30 minutes).
3. Once equipment has been disinfected, place “Decontaminated” sign on equipment to indicate the decontamination procedure has been complete and maintenance of equipment can occur.

## Autoclave

* All biohazard materials must be placed in appropriate biohazard containers and autoclaved before disposal. Anything that contacted cells should be disposed of here (paper towels, wrappers, etc.)
* Place tips of serological pipettes facing down.
* When bag is ¾ full, remove bag and replace.
* Avoid autoclaving bleach solution. If it is impractical to rinse items, the 10% bleach should be neutralized by adding 1 mL of 5% sodium thiosulfate per mL of 10% hypochlorite ion.
* See <http://bit.ly/autoclave-prep> and <http://bit.ly/autoclave-equipment> for reference.
* Items that cannot be autoclaved: oil; waxes; materials containing solvents, >3% chlorinated compounds (i.e. HCl, bleach), corrosive chemicals (i.e. phenol), flammable materials, & radioisotopes.

|  |  |  |
| --- | --- | --- |
| Plastic Type | Autoclave Compatible? | Number on Plastic |
| PETE or PET – Polyethylene Terephthalate  | no  | 1  |
| HDPE – High-density polyethylene  | no  | 2  |
| PVC or Vinyl – Polyvinyl Chloride  | no  | 3  |
| LDPE – Low-density polyethylene  | no  | 4  |
| PP - Polypropylene  | **yes**  | **5**  |
| PS - Polystyrene  | no  | 6  |
| PC - Polycarbonate  | **yes**  | **7**  |
| PE - Polyethylene  | no  | -  |
| PMP - Polymethylpentene  | **yes**  | **-**  |
| PTFE Resin  | **yes**  | **-**  |

### Liquid Waste (Autoclave)

1. Wear gloves, lab coat, pants, closed-foot shoes, and eye/ face protection.
2. No other types of waste should be mixed or included with contaminated liquid wastes.
3. Place contaminated waste in an autoclave-compatible container.
4. Inactivate waste by autoclave (121° C for at least 30 min). Inactivated waste can be treated as decontaminated and can be directly disposed down the sewer while being cautious to prevent the formation of aerosols or spills.

### Solid Waste (Autoclave)

1. Wear gloves, lab coat, pants, closed-foot shoes, and eye/ face protection.
2. Place contaminated waste in a biohazard/ autoclave bag, including: gloves, paper towels, microcentrifuge tubes, pipette tips, Kim wipes, plate covers, reagent reservoirs, pH strips, vials for standards and controls, microtitre plates, and straws – no other types of waste should be mixed or included with contaminated solid wastes.
* Do not autoclave bleach soaked paper towels—just rinse these thoroughly and throw them in a regular garbage
1. Inactivate waste by autoclave (121° C for at least 30 min). Inactivated waste can be treated as decontaminated and can be directly disposed of into a regular garbage bin. **Biohazard symbols and chemical labels on wares and bags must be defaced after being autoclaved**.

## Third-Party Disposal/ Incineration

1. If waste cannot go in autoclave or be mixed with bleach, waste can be collected and disposed of by third-party.
2. Wear gloves, lab coat, closed-foot shoes, pants, and other appropriate personal protective equipment, including face and eye protection.
3. No other items (i.e. solid) should be mixed with liquid waste (and vice-versa).
4. Place waste in properly labeled, leak-proof waste container (available from Science Stores). Fill container no more than 75% full.
5. If applicable, put chemical waste label on container - label all chemicals and biological materials.
6. Disinfect outside of waste container with 10% bleach.
7. Contact health.safety@uregina.ca for disposal.

## Human Tissues, Solids, and Items Saturated with Blood and Body Fluids

1. Wear gloves, laboratory coat, closed-toe shoes, and other appropriate personal protective equipment, including face and eye protection.
2. No other items (i.e. liquids) should be mixed with solid waste.
3. Place waste in a red biohazard pail available from Science Stores. Fill container no more than 75% full.
4. Put chemical waste label on container - label all chemicals and biological materials.
5. Disinfect outside of waste container with 70% ethanol.
6. Contact health.safety@uregina.ca for disposal.

## Sharps Contaminated with Human Materials (i.e. Needles, razor blade, scalpels, etc.)

1. Wear gloves, laboratory coat, closed-toe shoes, and other appropriate personal protective equipment, including face and eye protection.
2. No other items (i.e. liquids, solids) should be mixed with sharps waste.
3. Place waste in a biohazard sharps waste container available from Science Stores. Fill container no more than 75% full.
4. If applicable, put on chemical waste label and label all chemicals.
5. Contact health.safety@uregina.ca for disposal.

## Broken Glass Contaminated with Human Materials

1. Wear gloves, laboratory coat, closed-toe shoes, and other appropriate personal protective equipment, including face and eye protection.
2. No other items (i.e. liquids, solids, needled, intact glass) should be mixed with broken glass waste.
3. Place waste in a white “broken glass” waste container available from Science Stores. Fill container no more than 75% full.
4. If applicable, put on chemical waste label and label all chemicals.
5. Contact health.safety@uregina.ca for disposal.

# Biological Safety Cabinet Class II Type A1 Procedures

Biological safety cabinets (or biosafety cabinets; BSCs) Class II Type A1 are vented cabinets, in which the air drawn into the cabinet is passed through a High Efficiency Particulate Air (HEPA) filter before flowing downwards towards the work surface. The downward air captures the aerosol particles generated at the work surface, thereby providing the highest level of product protection. They differ from chemical fume hoods – **do not use a biosafety cabinet as a chemical fume hood.**

* Biosafety cabinets must be certified prior to use. A qualified third-party contractor must certify these cabinets annually. Check the certification sticker on the front of the unit to verify your hood’s condition.
* All repairs made on biosafety cabinets must be made by a qualified technician. Before any third-party maintenance (e.g. HEPA filter removal, annual maintenance, etc.), cabinets must be properly decontaminated (see Section #14 – Decontamination and Waste).
* Biosafety cabinets can be equipped with one of two kinds of alarms:
* **Sash alarms** are found only on cabinets with sliding sashes. This alarm signifies that the operator has moved the sash to an improper position. Corrective action for this type of alarm is returning the sash to the proper position.
* **Airflow alarms** indicate a disruption in the cabinet’s normal airflow pattern. This alarm represents an immediate danger to the operator or product and when an airflow alarm sounds, work should cease immediately and PI is notified.
* Training must be equipment specific and documented.
1. If biosafety cabinets are used not properly, their protective benefits may be greatly reduced. To ensure sterility inside the hood and establish proper air flow for product protection, the blower should be turned on at least 10 minutes before biological materials are to be put in the hood. As an additional method of surface sterilization, lower cabinet sash and turn on UV light for 5 minutes. **Remember to turn off UV lamp prior to work – you will get a UV burn**!!
	* If UV lights are used to decontaminate the work surfaces inside a cabinet, the 253.7 wavelength has limited penetrating power, and is only effective against microbes in the air or on the work surface.
	* The intensity of the lamp, and therefore, the ability of the lamp to sterilize, decreases with time.
	* The intensity of the radiation decreases as the square of the distance of the lamp; therefore, exposure time required is related to the distance from the lamp.
	* The lamp must be cleaned regularly.
	* The UV light reflects off the cabinet surfaces and is a risk to persons working in or near the cabinet. Never operate the lamp if a worker is near the cabinet.
2. The cabinet air flow gauge should be checked to assure proper operation of the cabinet before placing any materials into it (Read operating instructions). Readings indicate relative pressure drop across the HEPA filter. Higher readings may, therefore, indicate filter clogging. Zero readings may indicate loss of filter integrity. In either of these cases, notify the PI. NEVER remove or touch the HEPA filter yourself.
3. Put on nitrile gloves, laboratory coat, long pants, and closed-foot shoes.
	* Gloves pulled over the wrists of the coat rather than worn inside; double-gloves should be considered
	* Masks and safety glasses may be required for some procedures
4. The number of movements across the front opening should be minimized by placing all necessary items into the cabinet prior to beginning manipulations.
5. Wipe inner surfaces (especially the pan) with a solution of either 70% ethanol or 10% household bleach and allow to dry. Always keep a bottle of disinfectant (e.g., bleach, 70% ethanol, etc.) near the cabinet for decontaminating, or in case of a spill. Never keep the bottle in the cabinet, as the UV breaks down the plastic containers and lids.
6. **NEVER place anything over the front or rear grille of a cabinet.** Disrupting the air flow into the front grill allows non-sterile air from the room to blow into the cabinet over your experiments!
7. Materials should be placed in the cabinet so as not to block air flow into the rear grille. Leave a few inches for air to flow around things. Any disruption of the air flow in the cabinet decreases its effectiveness. Remember: **“A cabinet is only as safe as the person using it.”**
	* Aerosol generating equipment (e.g. mixers, centrifuges) should be placed towards the rear of the cabinet.
	* Bulky items such biohazard bags and discard pipette containers should be placed inside and to one side of the interior cabinet.
8. Before manipulating infectious materials, try to make sure that you have everything you need in the cabinet. The fewer times you pull your hands out of the cabinet, the less disruption of the air flow.
9. **Your arms should be moved in and out of the cabinet slowly, perpendicular to the front opening.** When you put your hands in the cabinet for the final time, move hands into the cabinet perpendicular to the glass and wait 1 minute before starting manipulations.
10. **Do NOT use gas burners inside biological safety cabinets.**
11. Work should be performed on the center of the work surface of the cabinet whenever possible. Work outward progressing from clean to dirty (contaminated). However, infectious agents should not be placed directly adjacent to or directly on the intake grills.
12. After manipulating infectious agents, make sure all are in tightly closed containers before removing them. Wipe down the surface of all equipment used in manipulations (pipettors, etc.) with disinfectant before removing from the cabinet. All waste and disposable items should be left in the cabinet until properly decontaminated or contained.
13. When removing hands or items, move hands out of the hood perpendicular to the glass and wait 1 minute to allow the air flow to resume.
14. After the cabinet has been emptied, wipe exposed surfaces including the front grille and splash area with 1 in 10 dilution of bleach followed by 70% ethanol. Allow the blower to run for a minimum of ten minutes to purge any aerosols from inside the cabinet before shutting off the blower.
15. The bleach in the vacuum traps must be changed after one week of use or when the flask is half full. To discard trap liquid, first treat with bleach for 20 minutes and then empty into the sink with large volumes of water.
16. The vacuum filters must be replaced if clogged or if liquid makes contact with the filter (see below SOP). Used filters should be placed in the waste to be autoclaved.

NOTE: Class IIA cabinets recirculate about 70% of the air inside themselves and exhaust the remainder to the lab. Any use of volatile solvents should be kept to a minimum or done elsewhere. Dangerously high levels of volatile fumes can accumulate inside the cabinet and pose a threat of fire or explosion.

# Centrifuge Procedures

### Ultra, High-Speed, and Benchtop Centrifuges

* Centrifuges must be inspected prior to each use to ensure gasket integrity. Inspect the rotor for signs of corrosion or cracking before using. Inspect the inter-lock system to ensure the cover cannot be opened while the rotor is spinning. Centrifuge may not be used if these items are compromised. This can be logged using **Appendix C.**
* Lubricate the o-rings and threads as recommended by the manufacturer. This can be logged using **Appendix C.**
* Rotors with o-rings (aerosol containment) must be used with RG2 material.
* Click-lids must be used on the ultracentrifuges at all times. If the click-lid is compromised/ will not lock the centrifuge is not to be used and PI to be notified.
* All centrifuges that have manufacturers’ rotor de-rating systems (ultracentrifuges and high-speed) must have an up-to-date record of total hours of usage. This is essential to prevent rotor fatigue. This can be logged using **Appendix C.** DO NOT USE a rotor after the expiration date (on some model marked on the rotor and rotor accessories); take these components out of service.
* The centrifuge should be securely anchored by strong suction cups (bench top models), wheel brakes (floor models), etc. Movement of the instrument can damage parts and injure users.
1. Centrifuges should always be installed according to manufacturer’s specifications – they should not be located near areas containing flammable reagents or combustible fluids, or where vibration will cause items to fall off nearby shelves.
2. Put on nitrile gloves, lab coat, long pants, and closed-foot shoes.
3. Inspect centrifuge prior to each use to ensure gasket integrity. This can be logged using **Appendix C.**
4. Ensure that the sample tubes or bottles are designed for the particular rotor used. Always cap samples tightly to avoid creating aerosols. Plastic centrifuge tubes should be discarded after one cycle of ultracentrifugation.
5. Never load a centrifuge unbalanced. Use a scale or other means of verification.
6. Do not leave centrifuge until full operating speed is obtained and the instrument appears to be running normally without vibration. Log use in **Appendix C.**
7. In event of a power failure, do not try to open the lid to retrieve samples for a least one hour. After the rotor has stopped, follow the instructions in the manual for recovery of the samples.
8. Allow five minute time for aerosols to settle before opening or removing the covers/ lids.
9. Always leave the drum of the centrifuge clean; wipe up any spills or aerosols. Do not use a bottle brush to clean the cavities of a rotor, as it may scratch the rotor, and allow corrosion to start. For spill procedures (see above Section 3).

# Emergency Procedures: Confirmed Prion Contamination

**In the extremely rare case that human neurological tissue is determined to contain prions/ TSE by RQHR, the following procedures must be followed:**

If at any time the RQHR verifies human neurological tissue that was given to the UofR contains prions/ TSE, immediately stop activities and phone Health, Safety & Environment at 306.526.6656.

## Prion Disinfection

* Prions are characterized by extreme resistance to conventional inactivation procedures including irradiation, boiling, dry heat, and many chemicals (formalin, betapropiolactone, alcohols). Fixation with alcohol, formalin, or glutaraldehyde strongly stabilizes the infectivity of prions and makes them more difficult to inactivate. Formalin-fixed and paraffin-embedded tissues, especially of the brain, remain infectious.
* As a consequence, contaminated materials should not be exposed to fixation reagents, and should be kept wet between the time of use and disinfection by immersion in chemical disinfectants. Fixed material that contain or may contain prions must be disposed of as "prion waste".

### Effective Chemical Disinfectants

The only effective chemical disinfectants to inactive prions include:

1. Freshly made 40% household bleach, per USDA requirements (1 part 5.25% bleach plus 1.5 parts water to produce 40% bleach solution, which equals to 20,000 ppm) for 1 hour
2. Freshly made 2N NaOH for 1 hour

### Disinfection of Surfaces and All Laboratory Equipment

1. Always wear double nitrile gloves, disposable lab coat, pants, closed-foot shoes, and face shield when handling prions and chemical disinfectant.
	1. Note: HSW has extra disposable PPE available:
		1. Booties/ Shoe covering
		2. Pants
		3. Full cover-alls
		4. Respirators
2. Flood all surfaces with 2.0 N NaOH or 40% household bleach and let stand for 1 hour.
3. For awkward surfaces/ sides/ etc. soak towels with 2.0 N NaOH or 40% household bleach and place over equipment. Keep wetting towels for 1.5 hour.
4. Mop up, wipe down, and rinse with water.
5. If possible, autoclave the equipment at 134°C for 1 hour. \*Must contact health.safety@uregina.ca to make arrangements.\*

### Biological Safety Cabinet Decontamination

Because the paraformaldehyde vaporization procedure does not diminish prion titers, BSCs must be decontaminated with 2N NaOH and rinsed with water. HEPA filters should be bagged out and incinerated. This must be completed by a certified third-party vendor only.

### Autoclave

The use of conventional autoclaves as the sole treatment has **not** resulted in complete inactivation of prions. The safest method for ensuing that there is no risk of residual infectivity on contaminated instruments and other materials is to discard and destroy them by incineration.

**\*\***Extremely important: **Must contact** **health.safety@uregina.ca** **to make arrangements before using the autoclave (2016-02\*\***

## Prion Spill Clean Up

Immediately notify other lab personnel that a spill has occurred. **It is very important to keep contaminated surfaces moist until decontamination is complete** as the infectious agents become even more resistant to chemical inactivation when dry.

### Small Hazardous Biological Spill

(Spills you are comfortable cleaning up)

1. All persons should immediately leave the affected area and allow aerosols to settle (~ 1 hr – 1 day).
2. Signs should be posted indicating that entry into area is forbidden. Post a sign stating “DO NOT ENTER, BIOHAZARD SPILL. Contact (name and phone #) for information.”
3. Any exposed person should seek medical assistance immediately (within 1-2 hours) from a health care professional.
4. PI, Health, Safety & Environment (306-585-4776), and/or a “Spill Buddy” should be informed for cleanup assistance.
5. Always wear double nitrile gloves, disposable lab coat, pants, closed-foot shoes, and face shield when handling prions and chemical disinfectant.
	1. Note: HSW has extra disposable PPE available:
		1. Booties/ Shoe covering
		2. Pants
		3. Full cover-alls
		4. Respirators
6. Cover the spill with cloth or paper towels to contain it.
7. Saturate/ flood paper towels with fresh 2.0 N NaOH or 40% household bleach, and let sit for at least 1 hour. Start applying the disinfectant from the outside and move inwards.
8. If possible leave the lab to avoid prolonged breathing of fumes. Direct others not to enter the lab.
9. After the appropriate amount of time (see PSDS), clear away any materials like broken glass using forceps or another mechanical device and place in a sharps container/biohazard container.
10. Clean and disinfect the spillage area using paper towels and other appropriate cleaning materials.
11. Place contaminated cleaning materials into a RED labelled, leak-proof, puncture-resistant waste disposal container and close container securely. Waste must be incinerated.
12. Wipe/ disinfect outside of container with fresh 2.0 N NaOH or 40% household bleach, and let sit for at least 1 hour.
13. Contact Health, Safety & Environment (health.safety@uregina.ca) for waste disposal assistance.
14. Complete an **Incident Report Form** and forward to Health, Safety & Environment within 24 hours. Forms can be found online [www.uregina.ca/hr/hse](http://www.uregina.ca/hr/hse) or by contacting health.safety@uregina.ca.

### Large Hazardous Biological Spill

(Spills you are not comfortable cleaning up)

1. All persons should immediately leave the affected area and allow aerosols to settle (~ 1 hr – 1 day).
2. Signs should be posted indicating that entry into area is forbidden; post a sign stating “DO NOT ENTER, BIOHAZARD SPILL. Contact (name and phone #) for information.”
3. Any exposed person should seek medical assistance immediately (within 1-2 hours) from a health care professional.
4. PI and UR Hazardous Material Spill Response Team (via Campus Security (306-585- 4999)) should be informed for cleanup assistance.

## Prion Waste

All waste must be incinerated by a third-party. Only place prion-waste in the RED labelled, leak-proof, puncture-resistant waste disposal container available from Science Stores.

### Third-Party Disposal/ Incineration

1. Always wear double nitrile gloves, disposable lab coat, pants, closed-foot shoes, and face shield when handling prions and chemical disinfectant.
	1. Note: HSW has extra disposable PPE available:
		1. Booties/ Shoe covering
		2. Pants
		3. Full cover-alls
		4. Respirators
2. No other items (i.e. solid) should be mixed with liquid waste (and vice-versa). Use a separate RED leak-proof waste container for liquid and solid materials.
3. Place waste in RED properly labeled, leak-proof waste container (available from Science Stores). Fill container no more than 75% full.
4. If applicable, put chemical waste label on container - label all chemicals and biological materials.
5. Wipe/ disinfect outside of container with fresh 2.0 N NaOH or 40% household bleach, and let sit for at least 1 hour.
6. Immediately, contact health.safety@uregina.ca for disposal.

### Sharps Contaminated with Prions (i.e. Needles, razor blade, scalpels, etc.)

1. Always wear double nitrile gloves, disposable lab coat, pants, closed-foot shoes, and face shield when handling prions and chemical disinfectant.
	1. Note: HSW has extra disposable PPE available:
		1. Booties/ Shoe covering
		2. Pants
		3. Full cover-alls
		4. Respirators
2. Place waste in a biohazard sharps waste container available from Science Stores. Fill container no more than 75% full.
3. Immediately place biohazard sharps waste container inside a RED properly labeled, leak-proof waste container (available from Science Stores). Fill container no more than 75% full.
4. If applicable, put chemical waste label on container - label all chemicals and biological materials (i.e., “Prion Waste”).
5. In addition, place “Sharps Waste” label on outside of container.
6. Wipe/ disinfect outside of container with fresh 2.0 N NaOH or 40% household bleach, and let sit for at least 1 hour.
7. Immediately, contact health.safety@uregina.ca for disposal.

### Broken Glass/ Intact Glass Contaminated with Human Materials

1. Always wear double nitrile gloves, disposable lab coat, pants, closed-foot shoes, and face shield when handling prions and chemical disinfectant.
	1. Note: HSW has extra disposable PPE available:
		1. Booties/ Shoe covering
		2. Pants
		3. Full cover-alls
		4. Respirators
2. Immediately place broken glass/ glass waste inside a RED properly labeled, leak-proof waste container (available from Science Stores). Fill container no more than 75% full.
3. If applicable, put on chemical waste label - label all chemicals and biological materials (i.e., “Prion Waste”).
4. In addition, place “Sharps Waste” label on outside of container.
5. Wipe/ disinfect outside of container with fresh 2.0 N NaOH or 40% household bleach, and let sit for at least 1 hour.
6. Immediately, contact health.safety@uregina.ca for disposal.

# References

Center for Disease Control and Prevention. N.D. *Section VII-H Prion Diseases*. Retrieved from: <http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_sect_VIII_h.pdf>

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University of Minnesota, Environmental Health & Safety. N.D. *Prion Research Procedures*. Retrieved from: <http://www.dehs.umn.edu/bio_pracprin_prions_sp.htm>

# Appendix A - Authorized Users

Only users listed are approved for entry into \_\_\_\_\_\_\_\_\_ without the permission of Dr. \_\_\_\_\_\_\_\_\_\_. All others must first be authorized by Dr.\_\_\_\_\_\_\_\_\_\_, and must sign the visitor sheet.

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# Appendix B – Authorized User Waiver and Training Documentation/ Checklist

## Authorization and Signature

We, the undersigned, agree to the rules set out in this document:

I acknowledge that the \_\_\_\_\_\_\_\_\_\_ Lab in \_\_\_\_\_\_\_\_\_\_ uses potentially biohazardous material, which can cause infections if the material comes into contact with open wounds, mucous membranes or eyes and is inhaled, ingested or injected.

I have notified Dr. \_\_\_\_\_\_\_\_\_\_\_ of any preexisting medical conditions that could render me immunocompromised, as this would put me at greater risk for infection.

I agree to take all appropriate safety training and renew training as required.

I agree to read all lab Safe Operating Procedures before undertaking any experiments in the laboratory.

I have located all emergency features of the laboratory (sink, shower, eyewash, fire extinguisher, phone, first aid kit, spill kits) and know where to find the SOPs, SDS/PSDS, and safety guides in the lab.

I agree to abide by the following general rules:

1. Lab doors must remain closed and locked at all times.
2. Follow all biosecurity requirements.
3. Only authorized users are permitted in the lab. All visitors showing up at the door must be sent to the PI.
4. Food and drink may only be stored and consumed in the office areas; lab coats, gloves, or other items from the lab are not permitted in the office area.
5. Horseplay is not allowed in the lab at any time.
6. Lab coats, gloves, pants, and appropriate clothing/footwear must be worn when handling potentially biohazardous materials.
7. Benches and wares must be wiped down with either 70% ethanol or a 10% bleach solution after culturing, transferring, or manipulating any biohazardous materials.
8. The biological safety cabinet must be used for all activities that create aerosols (i.e., centrifugation, pipetting, vortexing, etc.)
9. All biohazardous material must be autoclaved, treated with appropriate chemical disinfectant, or incinerated before disposal.
10. All vessels containing biological materials or waste must be labeled with the contents. No exceptions.
11. All material must be inventoried, if biological material remains in containers for more than 30 days.
12. Accidents, spills, or possible losses of containment must be reported to the PI.
13. Lab coats, gloves, and eye protection must be removed, and hands washed before exiting the lab.
14. A final walkthrough/safety check will be done as the last thing before leaving the lab each day.
15. Others' workspace and experiments will be respected and safeguarded at all times.
16. All self experiments are strictly prohibited.
17. When your research term has ended, your office desk, bench, and samples in refrigerators and freezers will be left clean, and organized, and lab coats will be autoclaved if being taken out of the lab.

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

Lab Member Name \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_, PI

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

Lab Member Signature Date

## Training Documentation/ Checklist

Date Training: Name of Trainer:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 Trained on the relevant physical operation and design of the containment zone and systems:

* Emergency eyewash and shower
* Fire extinguisher
* First Aid Kit
* Emergency gas shut-off button
* Spill Kits
* Telephone
* Clean and dirty areas; traffic flow

 Trained on the correct use and operation of lab equipment

* Biological Safety Cabinets
* Centrifuges
* Incubators
* Equipment creating aerosols
* Electrophoresis
* Bunsen Burners/ Natural Gas
* Sharps
* Microtomes
* Vacuum pumps and systems

**SOP Training Documentation**

Date Observed: Name of Observer:

Activities:

Demonstrates competency in handling microbes safely using proper technique, and working around a Bunsen burner and ethanol.

Demonstrates proper lab and safety etiquette, including the use of PPE (gloves and lab coat).

Demonstrates understanding of the boundary between clean areas and containment zones.

Demonstrates competency in proper disposal procedures, such as use of biohazard receptacles and autoclave for disposing of contaminated wastes.

Demonstrates competency in proper decontamination procedures.

Additional Comments:

# Appendix C – Laboratory Inspections/ Duties

**Lab Maintenance (Surface maintenance, cabinet wood sealing, roof repairs, etc.)**

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| **Date** | **Task** | **Solution/ Comments** | **Initials** |
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**Regular Lab Cleaning (All Cleaners Must Initial)**

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**Equipment Maintenance (Centrifuge seals, Rotor Cups Integrity, Gaskets, Freezer Filters, HEPA Filters, etc.)**

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| **Date** | **Task** | **Solution/ Comments** | **Initials** |
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**Regular Lab Inspection and Correction Actions**

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| **Date** | **Inspection Task** | **Correction Action Recommended & Date Complete** | **Initials** |
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