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1. Introduction

This document will outline the appropriate vessels and conditions for storing biohazardous material in the lab. All biohazardous material must be kept in a safe and secure location, and at appropriate temperatures. This material must be listed in an inventory file approved by the Queen's University Biohazard Committee. This file must be available in the lab on an accessible computer/shared drive or printed and posted on the fridge/ freezer/dewar. If they are located in a shared space, the storage equipment must have lab contact information posted.

There is also information regarding storage conditions of biohazardous samples. This includes maintenance and cleaning of fridges, cold labs and cold storage rooms.

2. Scope

This SOP is for all labs with biohazardous samples and an approved biohazard permit for work in containment level 1, 2 and 2+ laboratories.

3. Applicable Legislation, Standards, Guidelines:

Canadian Biosafety Standards, 3rd Edition, 2022

4. Responsibilities

4.1 Responsibilities of Directors, Department Heads and Managers

- Each has the following responsibilities under this standard operating procedure.
- Ensure that a written assessment of potential hazards has been completed for all areas under his/her authority.
- Ensure that this SOP is implemented in all facilities under his/her authority.
- Ensure that all pertinent supervisors, employees and students are aware of this SOP and have been informed of the proper use, care and maintenance of storing biohazardous samples.

4.2 Responsibilities of Supervisors

- Supervisors must be knowledgeable about the hazards in their area. They must:
- Ensure that all staff and students are aware of the hazards present and have been informed of the proper use, care and storage of biohazardous materials.

- Ensure that workers follow this SOP
- Ensure that the Biosafety Officer is informed of any lost or stolen biohazardous material. Also, they must be made aware of any spills, exposure or incidents related to this material.

4.3 Responsibilities of Staff and Students

- Be familiar with the content of this SOP.
- Complete the required safety training before any work with approved biohazardous material begins.
- Adhere to all safety precautions specified in this SOP.
- Report any issues, hazards, or concerns to a supervisor.

5. Definitions

Cold lab
A cold room that is used for storage and manipulation of biohazardous samples. These rooms will have a biohazard containment level posted on the door.

Cold storage room
A cold room that is used for storage only. No biohazard samples are manipulated in this area.

Storage vessel
Any item that is used to store a sample with a lid, tight-fitting enclosure or door.

Secondary Containment
Any device designed to prevent the spread of hazardous material spilled from its primary storage (containment) vessel.

6. Safety precautions

Refer to the specific Pathogen Safety Data Sheet (PSDS) and Standard Operation Procedures (SOPs) related to biohazardous material being manipulated or stored. Basic PPE includes gloves and lab coat, however additional PPE may be required (such as double gloves, face shield etc).

If your fridge, cold lab or cold storage room is contaminated with mold, contact EHS. It is not recommended to remediate large moldy areas on your own. Refer to Mold Prevention Assessment and Remediation Procedures SOP-CHEM-04.

7. Work practices

Storage Vessels

All vessels must be properly labeled. If it is stored within the lab, follow your lab's labeling system. If stored in a shared location, include your lab name along with sample and date information. If using a coding system, ensure this is available to your lab members. Secondary containment is required for all samples stored in shared locations, or outside of containment.

Storage vessel	Storage parameters
Culture plates	-Sealed with parafilm (individual plates) -Stored in plastic sleeve/bag, secured with tape or stored in plastic container (secondary containment) -Label individual plates, bag or plastic container.
Culture flasks	-Lids/caps can be loosened to allow for aerobic conditions, but must be secure -Stored flasks (not shaking) must be in secondary container such as plastic tray. -Stored on bench or shelf, secured -Label flasks
Culture tubes (conical, snap cap)	-Stored in boxes or plastic containers -Lids/caps can be loosened to allow for aerobic conditions, but must be secure -Label samples -If tubes are too small to write enough information, use a coding system.
Hinge/screw cap tubes/cryo vials	-Stored in racks or boxes -Lids must be closed -If tubes are too small to write enough information, use a coding system.
Cardboard/plastic sample boxes	-Clean, dry storage

	-Closed with lid -Securely stored (as not to fall off bench or shelf)
Liquid nitrogen dewar	-Must be stored in a well-ventilated area -Dewar must be monitored to ensure it does not go dry (with a sensor if possible) -Biohazard symbol should be posted on the dewar if it is stored outside of containment.
Fridges/Freezers/Dewars	-Must display a biohazard symbol if storing biohazardous material outside of containment.

Storage conditions

It is important to keep a well maintained and secured storage area for your biohazardous samples. Creating a regular cleaning schedule will help reduce mold and other contamination, especially in shared storage spaces.

Room temperature (open bench)

- Ensure the samples are stored in the appropriate vessel.
- Must be stored away from the edge of the shelf or bench, or where it can't get knocked over easily.
- Recommended to make a designated room temp storage area, so cultures and tubes aren't lying around on every bench.
- Check the integrity of the samples periodically.

Refrigeration (including cold storage)

- Ensure the samples are stored in an appropriate vessel (i.e. use plastic containers/boxes instead of cardboard)
- The temperature should be easy to read. If the fridge does not have a built-in temp display, it is recommended to keep a thermometer inside.
- Disinfect and clean out the fridge on a consistent schedule. Be sure to wipe down the gaskets around the door as well, to help maintain a good seal.
- Clean up all spills immediately to help prevent mold and contamination.
- Cold rooms now have two designations and will have appropriate signage:
    - ***Cold storage rooms***, which are not required to be listed on your permit for inspection, but your inventory must include storage location.

- ***Cold labs***, which are required to be listed on your permit, and are subject to annual inspection. Biohazardous sample manipulation is allowed in these rooms, along with incubators, centrifuges and shakers.

Freezer (-20C or -80C)

- Ensure the samples are stored in an appropriate vessel that can withstand freezing.
- The temperature should be easy to read. If the freezer does not have a built-in temp display, it is recommended to keep a thermometer inside.
- Ultra low's (-80C) must be monitored with a temperature alarm.
- Keeping a daily temperature log for any -80˚C freezer is highly recommended for quality assurance purposes, i.e., to monitor the range of daily fluctuations, for malfunctions/ error messages, and for trends of deteriorating performance. This is usually an automatic feature.
- Wear appropriate PPE to protect your skin from frost bite when entering ultra-low temperatures (protect skin with lab coat, long sleeves, gloves).
- Keep a diagram of exactly where you store materials in the freezer so you can go right to what you need, as opposed to searching randomly with the freezer door open and allowing the contents to warm.
- Try to coordinate with coworkers to access freezer contents in one or two trips to avoid multiple freezer openings throughout the day.
- Freezers cannot be decontaminated unless they are defrosted first.
- -20C freezers typically do not defrost on their own, to maintain a consistent temperature. These must be periodically thawed and cleaned to remove excess ice buildup.
- It is not recommended to defrost an ultra-low unless necessary. These freezers are typically cleared of extra frost by scraping the excess out.
- Please see the defrosting procedure at the end of this document.

Liquid Nitrogen Dewars

- Ensure the samples are stored in an appropriate vessel, that can withstand extreme freezing.
- Dewars must be stored in a well-ventilated area, to prevent asphyxiation.
- It is recommended that they have a monitoring system on them, so they don't run dry and compromise your samples.
- Proper PPE must be worn when accessing the dewar, such as cryo gloves, face shield and lab coat, proper footwear, cryo apron.

Defrosting, Scraping and Decontamination

Defrosting and scraping of -20˚C and -80˚C freezers are done to reduce the amount of ice buildup and increase the efficiency and longevity of these storage vessels. Decontamination is done to these storage vessels when a sample breaks primary containment.

Defrosting Procedure for -20C

- Schedule a -20˚C freezer defrost procedure with plenty of lead time in which to plan where you can relocate your inventory. -20˚C storage space in labs is usually at a premium, so finding extra space to temporarily house your inventory may be difficult. Ensure you use a secondary container labelled with a biohazard symbol on a cart to move your samples, to contain any possible melting ice or samples that have been compromised.
- Expect the entire process to take two days.
- Wear appropriate PPE for this process: thermal insulated (cryo) gloves, lab coat and face shield. PPE should also be determined based on the biohazard material stored in the freezer. Remove your inventory from the freezer in an organized way so that you can return it to the -20˚C in good order. Take advantage of this opportunity to discard any inventory that is no longer needed. If you no longer require an item listed in your biohazard inventory file associated with your permit, submit an amendment and mention what items were destroyed and how.
- When your inventory is secure elsewhere, power down the freezer and place a sign on it that warns others: “Out of Service – Defrosting in Progress – Unit Will Be Decontaminated for Biohazardous Materials by: (your name) Before Return to Service.” This sign can be found in Appendix B.
- Prop the door open to let thawing begin; a floor fan blowing room air into the freezer increases thawing rate. Expect complete thawing to take several hours.
- DO NOT USE SHARP OBJECTS TO CHIP ICE OUT OF THE FREEZER TO HASTEN THE PROCESS – THIS CAN DO STRUCTURAL DAMAGE TO THE FREEZER. Place pans on the shelves to catch melting ice. If the freezer stores biohazardous material, add one part bleach to 9 parts melted ice, and after 30 minutes contact time discard the meltwater down lab sink with running water.
- Place pan(s) on floor to catch the majority of meltwater. Place paper towels under the pan(s) and on floor around the freezer to catch and absorb any meltwater that does not go into the pans. As towels get wet, discard them in solid biowaste for autoclaving and replace them with dry towels.

Decontaminate damp floor area with a freshly prepared 1:10 dilution of bleach or other appropriate disinfectant.

- When ice is gone, decontaminate interior and exterior surfaces of the freezer with a freshly prepared 1:10 dilution of bleach or appropriate disinfectant, and allow to dry fully. To protect stainless steel surfaces, consider a quaternary ammonium disinfectant. Wipe any corrosive disinfectant residue (e.g., bleach) off with distilled (not deionized) water. Remember to replace your gloves throughout the process as needed.
- If accessible, clean dust and dirt off the exterior coils on the back of the freezer.
- Disinfect door handles during this process, and on a frequent basis.
- Turn the power on and allow freezer to come to temperature. Leave freezer empty for at least a day to ensure that it will attain and hold temperature before returning inventory to it.
- Plan the re-installation of inventory carefully so that it can be accomplished in as few sessions as possible, and as quickly and orderly as possible.
- Keep a close check on the freezer after inventory has been returned to ensure that the freezer keeps holding its temperature.

Scraping Frost from -80C freezers

Some departments have a preventative maintenance package set up with an external company that scrapes the -80C freezers on a set schedule. If that is the case, you will not need to follow these steps. However, if a sample is compromised in the freezer, and it was not in secondary containment, the freezer is considered contaminated. It must be shut down, thawed completely, decontaminated, cleaned and brought back in service (you can follow the steps for -20C freezer defrosting).

- Notify lab members of this process before it begins. Attach ‘Out of Service – Defrosting in Progress – Unit Will Be Decontaminated for Biohazardous Materials by: (your name) Before Return to Service. e’ sign from Appendix B.
- Wearing appropriate PPE (lab coat, cryo gloves, face shield), remove samples from the freezer and relocate them temporarily to another -80C freezer. A -20C freezer will not maintain the temperature of your samples. You can keep your freezer on during this process.
- Find bins, trays or plastic containers to collect frost during the scraping process.

- Start scraping the frost from each shelf of your freezer and around the gasket, collecting it in your bins. Add 100ml of concentrated bleach for each ~900ml of frost (meltwater at this point). Allow to sit for 30 min before pouring it down the drain. Change PPE as needed throughout this process.
- Remember to decontaminate the door handle during this process, and on a frequent basis.
- Close the -80C freezer and allow the temperature to drop back down before moving your samples back.

Liquid Nitrogen Dewar contamination

- If a sample tube opens or explodes in the dewar, it is considered contaminated.
- Wearing appropriate PPE, transfer all samples to another dewar and allow the liquid nitrogen to evaporate (in an area where asphyxiation will not occur). Make sure other users are aware that a dewar has been left open and is considered contaminated by posting a sign on the room or dewar itself. This process can take up to two days.
- Decontaminate the dewar with 10% bleach solution and allow it to sit for 30 min.
- Check manufacturers' recommendations for the type of detergent that can be used. Usually, a mild household detergent is ok. Rinse the dewar thoroughly with water.
- It is also recommended to wash the outside of the dewar occasionally as well, to prevent corrosion.
- Once the dewar has been cleaned, invert it to allow it to dry completely overnight before refilling it with liquid nitrogen.

Fridge, Cold Room Storage & Cold Lab Cleaning & Maintenance

Please find a cleaning & maintenance log in Appendix A that can be used for cold labs, cold storage rooms and refrigerators that store biohazardous samples. It's important to maintain a regular cleaning schedule to prevent contamination of your samples, and to protect workers from possible exposure to leaks and spills. When cleaning the area, also check the integrity of the unit.

The following items should be visually inspected during the maintenance check portion:

- Check for any leaks, condensation or mold growth.

- On fridges, check the gasket seal around the door. It should be intact and not falling off or cracked.
- The door should close easily.
- The temperature should be maintained at 4°C. If there is no temp display, it is recommended to have a dedicated thermometer in your fridge/cold room to monitor the temperature.

The following items should be cleaned on a regular basis (we suggest monthly):

- Wear appropriate PPE such as gloves, cryo gloves, lab coat and face shield.
- All items should be removed from the fridge or cold lab before cleaning. Ensure you find somewhere to store all the material before you begin cleaning and notify all lab members.
- Discard any expired or unnecessary products appropriately.
- Use cleaning solutions as directed by the manufacturer, avoiding harsh or corrosive chemicals that could damage the interior.
- Thoroughly clean all internal surfaces, such as shelves, drawers and gaskets, with a gentle disinfectant. Focus on removing any spills or stains, which can breed bacteria and disrupt temperature control.
- Wipe the door handles frequently.
- Ensure the fridge is completely dry before refilling.
- Opening fridge doors for prolonged periods can cause moisture to enter the cavity, which can lead to further issues with ice build-up. Consider turning off the refrigerator (not a cold room or cold lab) while it is empty to melt any ice build-up. Although most lab fridges have auto-defrost functions, regular ice checks and manual defrosting as needed are essential, as ice formation can reduce the fridge's efficiency.
- Allow the fridge to return to temperature before putting your items back.

8. Training requirements

You must complete the Biosafety Level 1, Biosafety Level 1 & 2 or Human Bloodborne Pathogens course in onQ depending on your Biosafety Training needs as outlined in your biohazard permit. Anyone working with biohazardous materials must be familiar with the content of this SOP.

9. References

CBS 3rd Edition, 2022
Queen's Biosafety Manual 2021
George Washington University Cold Room Guidelines 2018
University of Utah, EHS, Fact Sheet Storing Biohazardous Materials

LabRepCo: How to Clean a Liquid Nitrogen Dewar or Tank: Proper Sanitization & Decontamination

10. Revision History

Date	Revised by	Changes
May 2025	Created by Natalie Roy	First release

11. Appendix

Appendix A Cleaning Log for Fridges & Cold Storage/Labs

Name	Date	Maintenance check	Cleaned	Notes

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Appendix B

Out of Service

Defrosting in Progress

Unit Will Be Decontaminated for Biohazardous Materials by: _____ Before Return to Service