

**LOCAL JOINT HEALTH AND SAFETY COMMITTEE  
DEPARTMENT OF BIOMEDICAL SCIENCES  
STANDARD OPERATING PROCEDURE**

**1. WORKING WITH LIQUID NITROGEN, Dry Ice, and UltraCold Freezers**

**Effective Date:** April 8, 2011

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**Purpose:** To promote safe and proper handling of liquid nitrogen and samples stored in liquid nitrogen, dry ice and -80 freezers.

**Approvals required:** Faculty Supervisor, Local JHSC, EHS

**2. DEFINITIONS:**

**LN2:** Liquid nitrogen, -185°C liquid

**Dry Ice:** Solid carbon dioxide, at a temperature of -78°C or less.

**Ultracold Freezer:** -80 freezer

**PPE:** Personal Protective Equipment

**Sublimate:** Going from a solid to a gaseous state without passing through a liquid phase

**3. REQUIREMENTS:**

All persons must have WHMIS training and have completed departmental safety orientation.

**Personal protective equipment (PPE) is required at all times: lab coat, insulated gloves, safety glasses or full-face shield, and proper footwear (no open-toed shoes or sandals).**

**4. TASKS:**

**Dispensing Liquid Nitrogen**

- Only trained personnel are allowed to dispense LN2 from the bulk(160L) tank. Follow precautions for all compressed gases when handling the large LN2 tank.
- Liquid nitrogen is very cold (-185°C). Always protect your skin and eyes. **Use required PPE** including a full face shield when dispensing LN2 from large tank.
- When LN2 evaporates, gaseous nitrogen can displace oxygen in the environment, so keep the door open when dispensing LN2. If you feel any signs of O2 deficiency (rapid breathing, dizziness etc), turn off the valve and leave the room immediately. Do not dispense LN2 from bulk tank while alone in the lab. Asphyxiation can occur quickly in poorly ventilated areas - atmospheres with less than 19.5% O2 are unsafe.
- The dispensing hose is attached to the tank by the Linde delivery person. The regulator is equipped with a pressure release valve as part of its safety design. A tank may undergo frequent pressure release during initial temperature changes from outside to indoors. This is normal, however the sounds may be alarming to untrained personnel. Occasionally the valve may ice up when not closed properly. This can be rectified by using a warm towel to melt any ice built up at the valve. The valve can then be closed completely.
- After dispensing LN2, close the valve securely and leave the hose extended until it returns to ambient temperature. The hose may crack if bent while cold.

- Fill storage dewars only to the base of the neck. Overfilling can result in damage to the tank's insulation. Storage dewars should be monitored regularly. A leaking tank can release nitrogen gas into the atmosphere. In an enclosed space, asphyxiation of personnel could result. Caps on the dewars must fit loosely in order to prevent build up of pressure - which could result in an explosion.
- LN<sub>2</sub> dewars should not travel on a sealed elevator car with you. In case of power failure, the amount of nitrogen gas released in an enclosed space could be very dangerous. If dewars have to be moved on an elevator, it should be done by 2 people, with the tank travelling alone between floors.
- Liquid nitrogen should not be transported in a motor vehicle. Use a "dry shipper".

### **Storage and Retrieval of Samples in Liquid Nitrogen:**

- a) **Use required PPE. Warning: Containers stored in LN<sub>2</sub> can explode upon warming. Use only proper cryogenic tubes – never use eppendorf tubes to store specimens in LN<sub>2</sub>.**
- b) Remove the lid of the LN<sub>2</sub> container and set to one side. Very slowly, using the metal hook over the neck of the container, move the canister or rack to the centre.
- c) Lift and remove the canister slowly, allowing excess LN<sub>2</sub> to run back into the container.
- d) Make sure you know how your tank is designed so that you can avoid excessive warming of stored samples. If you drop a sample or cane into the tank, retrieve it immediately. Use a log-book so you know where your samples are and so that they can be located and removed quickly.

### **Use of LN<sub>2</sub> for Freezing/Thawing of Samples to Lyse Cells:**

- a) **Use required PPE. Use only LN<sub>2</sub> approved containers. Unsafe containers may explode!** The Dewar flask used for liquid nitrogen should be pre-cooled in the -20°C freezer.
- b) Remove the lid from the small LN<sub>2</sub> holding tank and slowly pour liquid nitrogen into the Dewar flask or LN<sub>2</sub> carrier. Protect your back from injury if the tank is heavy. Be careful to avoid splashes of LN<sub>2</sub>.
- c) Always carefully add and remove samples from the liquid nitrogen using tongs or forceps and ensuring that your face is protected. **Warning: Tubes in LN<sub>2</sub> may explode when warmed suddenly. ONLY USE CRYOTUBES FOR THIS PROCEDURE. Eppendorf tubes may be used for flash-freezing, if they are dipped with the cap open into the LN<sub>2</sub>! After removing from LN<sub>2</sub> the frozen samples can be capped and quickly transferred to the -80 freezer).** When finished, left-over LN<sub>2</sub> may be returned to the holding tank or allowed to evaporate in a fume hood.

### **Receipt, Shipment and Storage of Samples Packed on Dry Ice:**

- a) Shipments of samples packed in dry ice must be identified by warning labels as required by the **Transport of Dangerous Goods Act**.
- b) Shipments on dry ice generally include an inner styrofoam cooler. Carefully remove any sealing tape securing the cooler lid.
- c) **Use required PPE.** If the shipment is near the surface of the dry ice retrieve it using gloves or tongs. If the shipment is under the dry ice, carefully transfer loose dry ice into another styrofoam container until the shipment is exposed and can be removed.

d) Read the storage directions on the shipment (either  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  freezer) and immediately transfer the item to the appropriate freezer. Leftover dry ice can be stored in the  $-80$  freezer or left to sublimate in a fume hood.

**Safety Note: Under no circumstances should dry ice pellets be sealed inside a container such as plastic centrifuge tubes. As the dry ice pellet warms, release of gaseous  $\text{CO}_2$  will cause dangerous explosion of the tube.**

### **Storage and Retrieval of Samples in the Ultracold ( $-80$ ) Freezer:**

- a) **Use required PPE: Temperatures of  $-76^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$  can quickly cause frostbite and samples stored at these temperatures can damage the skin if handled with bare hands. Samples in eppendorf tubes may explode when warmed rapidly from  $-80\text{C}$ .**
- b) Use a log book so that you know where to locate your samples before you open the freezer door. Work quickly as the temperature in the interior of the freezer rises quickly.
- c) Open the appropriate inner door (one for each shelf) and remove the required storage box. Quickly close all doors making sure the outer latch closes properly. Place or remove your sample in the storage box and return it to the same location in the freezer. On leaving, make sure that the freezer doors are properly closed and latched in place.

### **5. CONTINGENCY PLAN AND REPORTING:**

If your skin comes in contact with  $\text{LN}_2$ , dry ice, the interior of the  $-80$  freezer or its contents, frostbite may result. Do not rub skin. Place under lukewarm but not hot running water and allow water to warm skin. Seek medical attention if necessary. Fill out an Injury/Incident report if medical attention is warranted. If  $\text{LN}_2$  or dry ice comes in contact with the eyes, flush with water at an eyewash station and immediately seek medical attention (call ext 2000 for help).

**In the event of a spill, evacuate personnel from the area for at least 30 minutes. For spills of  $\text{LN}_2$  greater than 4L, evacuate the lab and contact EHS for air testing prior to re-entry.**

### **6. WASTE MANAGEMENT:**

Allow  $\text{LN}_2$  and dry ice to evaporate or sublimate inside a fume hood.

**7. REFERENCES:** University of Guelph Safety Policy 851.08.05

### **8. DISTRIBUTION OF COPIES:**

Technicians, Graduate students, Project Students, other University of Guelph employees working with  $\text{LN}_2$ , dry ice or  $-80$  freezers.

Faculty Supervisors

Environmental Health and Safety

Local JHSC, Department of Biomedical Sciences

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**Authorization:** Faculty Supervisor

**Date:**