



Standard Operating Procedure

Handling and Transporting Tissue Samples Liquid Nitrogen (LN₂)

I. POLICY: The Puerto Rico Clinical and Translational Research Consortium (PRCTRC) ensure the safety and well being of participants in research studies performed in its facilities.

II. PURPOSE: This Standard Operating Procedure (SOP) details how LN₂ is transported from the laboratory to the clinic, how tissues are frozen in LN₂, and how they are transported back to the laboratory for storage in certain clinical research trials. This procedure is intended to ensure that LN₂ be handled in a safe and efficient manner while eliminating all possible risks and safety hazards.

III. Area(s) of Responsibility: This policy applies to the Principal Investigator (PI) and all the personnel responsible of handling liquid nitrogen.

ROLES:

Principal Investigators: The PI has the primary responsibility to ensure that all laboratory/research personnel are properly trained in and follow this SOP. This involves the following:

1. Preparation, proper storage, transfer and use of liquid nitrogen;
2. Provide appropriate Personal Protective Equipment (PPE) to employees and students where applicable;
3. Ensure that staff handling liquefied gases are instructed and trained as to the nature and safe handling of liquid nitrogen;
4. Require that all safety procedures be implemented.

Users: Personnel that will be handling liquid nitrogen are required to:

1. Adhere to safe handling procedures and safety precautions;
2. Wear appropriate Personal Protective Equipment (PPE);
3. Attend Lab Safety and required training sessions.

IV. Procedures:

All laboratory personnel should be in compliance with the Office for Safety in Research Laboratories (OSLI) and the UPR-MSU Employee Health and Safety regulations when working in the laboratory.

Materials, equipment and forms: The materials, equipment and forms listed below are required for the procedure.

Personal Protective Equipment (PPE)

The following Personal Protective Equipment (PPE) is required when handling liquid nitrogen:

1. Face shield that protects eyes and face;

2. Insulated cryogloves (should be loose fitting in case they need to be removed if liquid pours into them);
3. Splash resistant laboratory coat to minimize skin contact;
4. Closed toe shoes that cover the top of the foot or boots with trousers extended over the top of the boots to prevent liquid nitrogen from spilling into them;
5. Watches, rings and other jewelry should not be worn.

Storage

1. Liquid nitrogen should be stored and handled in containers specifically designed for the pressure and temperature to which they may be subjected;
2. Containers and systems containing liquid nitrogen should have pressure relief mechanisms. Tightly sealed containers will build up pressure as the liquid boils and may explode after a short period of time;
3. Do not store liquid nitrogen in uncovered containers for long periods;
4. Use cryogenic liquid dewars to store and transport small amounts of liquid nitrogen.
5. The dewar must be non-pressurized, vacuum-jacketed with a loose fitting cap to prevent air and moisture from entering while allowing excess pressure to vent.

Handling and Transportation: In view of the potential risks in handling liquid nitrogen, it is mandatory that when such operations are carried out, that at least one other person is in attendance or at least in the near vicinity. A second person in attendance should be able to provide assistance if required and take the appropriate action should an incident occur.

Transportation of LN₂ from the Laboratory to the Clinic

- a. Study coordinator will contact laboratory personnel previous to the date on which LN₂ shall be used;
- b. LN₂ should only be handled and transported by qualified study coordinator or authorized research personnel;
- c. LN₂ will be loaded into a specialized Dewar and sealed by a qualified laboratory technician;
- d. Proceed to transport the LN₂ Dewar to the clinic or the PRCTRC;
- e. Identify the cryovials with subject's ID number;
- f. Retrieve tissue samples according to the protocol provided by the researcher;
- g. Seal the cryovials once the samples are obtained;
- h. Wear the face shield, safety shoes and use protective insulated gloves. Use tongs to place the cryovials in the LN₂ gently to minimize boiling and splashing, then seal the dewar.

Transportation of LN₂ from the Clinic to the Laboratory

- a. Take the LN₂ dewar back to the laboratory. A qualified laboratory technician will receive it;
- b. The laboratory technician will proceed to open the dewar using the required PPE and retrieve the cryovials from the LN₂ and place them in the freezer.

Safety: Spillage

1. Alert people in the immediate area of spill;
2. If spill is in a laboratory ensure the doors are closed;
3. Keep all unnecessary personnel out of the area;
4. If the spill is in a hallway or running under the door, call OSLI at (787) 758-2525 ext.1687 and 1688 and (787) 766-3062;
5. Attend to contaminated personnel and remove from exposure;
6. If the person presents the symptoms of asphyxiation, such as dizziness or loss of consciousness while working with liquid nitrogen get a well-ventilated area immediately;
7. In case of a cryogenic burn caused by the LN₂ warm area of the body affected rapidly by immersing in water not exceeding 40°C, with body heat or by exposure to warm air. Do not rub or massage the affected part;
8. In the case of massive exposure remove all clothing and proceed to use the emergency shower. Maintain the affected area at normal body temperature until medical assistance arrives;
9. In case eyes are affected by the LN₂ flush eyes with warm water for 15 minutes;
10. When seeking medical assistance in case of an accident take a copy of the MSDS to the physician;
11. All incidents must be reported to the Laboratory Management or occupational office.

For assistance call:

- *MSC Office for Safety in Research Laboratories* at (787) 758-2525 ext. 1687 or 1688 and (787) 766-3062;
- *MSC Occupational Health Clinic* from Monday to Friday (8:00am-4:00 pm) call Ms. Juanita Rivera at (787) 758-2525 ext. 2913;
- Monday to Friday from 4:00pm to 11:00 pm - go to the Industrial Hospital Emergency Room;
- Saturday, Sunday and Holidays go to ASEM Emergency Room.

V. Precautions:

1. Splashing may occur when liquid nitrogen is dispensed into a warmer container or when cryovials are dipped into it. It may also occur when a substance at normal room temperature is inserted into the liquid nitrogen;
2. Dipping a hollow tube into liquid nitrogen may spurt liquid resulting in injury;
3. Liquid nitrogen is extremely cold and can cause severe frostbite or eye damage upon contact;
4. Unprotected body parts should not come in contact with vessels containing liquid nitrogen;
5. Sealed or insufficiently vented container can cause a dewar explosion;

6. Do not leave uncovered for an extended period of time, can result in reactions with organic materials (clothing fire may result);
7. Liquid nitrogen can displace sufficient oxygen if released in a confined space, making the atmosphere impossible for life sustention and causing asphyxiation/suffocation without warning. Entering an oxygen deficient atmosphere may cause unconsciousness without warning and death without regaining consciousness.