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1. OBJECTIVE

This SOP provides the procedures for safe handling of liquid nitrogen in Department of Medicine research laboratories.

2. SCOPE

This SOP is applicable to all staff and students in Department of Medicine research laboratories at MD1, MD6 and NUH.

3. RESPONSIBILITY AND ACCOUNTABILITY

3.1 The principal investigator (PI) is responsible to ensure that their staff and students are aware of the risk assessment and are trained for the safe handling of liquid nitrogen. The PIs shall ensure that necessary safety equipment is available in the laboratory.

3.2 All staff and students using liquid nitrogen in the laboratory must be aware of the potential hazards, and must be trained in the practices of such material safely.

4. PROPERTIES & HAZARDS

Liquid nitrogen (LN2) is inert, colorless, odorless, non-corrosive, non-flammable, tasteless, extremely cold, and has no warning properties.

The two properties of LN2 that present potential hazards are:

a. It is extremely cold - At atmospheric pressure, LN2 boils at -196 °C.
b. Small amounts of liquid vaporise into large amounts of gas. One liter of liquid nitrogen becomes 0.7 m^3 of gas.

The hazards arising from the use of LN2 are:

a. Cold burns and frostbites - Extremely low temperatures can freeze flesh very rapidly.
b. Asphyxiation - When the oxygen concentration in the air is sufficiently low, a person can become unconscious without any warning symptoms.
c. Explosion - On vaporization it expands by a factor of 700; one liter of LN2 becomes 24.6 cubic feet of nitrogen gas. This can cause explosion of a sealed or insufficiently vented container.
d. Cryotube explosions - Cryotubes used to contain samples stored under LN2 may explode without warning. Tube explosions are thought to be caused by LN2 entering the tube through minute cracks and then expanding rapidly as the tube thaws.
e. LN2 must not be disposed of down the drain, as piping in laboratory sinks may not be able to withstand cryogenic temperatures.

5. PERSONAL PROTECTIVE EQUIPMENT (PPE)

The following safety equipment is required when working with or dispensing LN2:

a. Face shield / safety glasses
b. Cryogenic gloves
c. Long-sleeved lab coat
d. Closed-toed shoes
e. Cryogenic apron

6. ENGINEERING CONTROLS

Adequate ventilation is essential when working with liquid nitrogen because a small amount of liquid can rapidly convert to a large volume of gas. Do not use in confined spaces because of the threat of asphyxiation.

7. LIQUID NITROGEN CONTAINERS

7.1 Vacuum insulated containers:

Vacuum insulated containers are used for storing and dispensing liquid nitrogen. They are either sealed (capable of holding a 20 psig – 240 psig with pressure relief valve) or ambient pressure (covered loosely with a cap, cork, or stopper) and are referred to as dewars. There are two primary types of dewars: benchtop and large dewars.

Benchtop dewars (Figure A) are typically for small-scale laboratory use and the lid is the only pressure-relief device.

Large dewars (Figure B) are typically used for storage of lab samples, movement of samples between campus locations, or to fill other secondary containers. Large dewars may have a secure seal and pressure relief vent.

Figure A– Benchtop Dewar

Figure B – Large Dewar

7.2 Cryogenic Tubes:

Cryogenic tubes are typically used for storage of samples. There is no pressure-relief device on a cryogenic tube other than the lid. Consequently, cryogenic tubes can explode with warning. Explosion are likely caused by trapped nitrogen expanding inside of the tube during the thawing process. As the temperature increase, the tube may become over-pressurized and explode and may result in serious injuries.

7.3 Self-Pressurizing Tanks:
Self-pressurizing tanks are generally a 140 – 260L double wall, stainless steel tank used to fill other liquid nitrogen containers. These tanks are equipped with pressure relief valves and a backup rupture disk. A loud hissing sound is commonly heard when the pressure relief valve opens. Exposure to liquid nitrogen can occur when connecting and disconnecting equipment, during the filling process, from a leaking valve, or from condensate ice buildup on valves and hoses.

8. SAFE HANDLING AND STORAGE

a. Only trained personnel should work with liquid nitrogen.
b. Use only in well-ventilated and low traffic areas.
c. Wear proper PPE: long sleeved lab coat, covered-toe shoes, cryogenic gloves, safety glasses, face shield and cryogenic apron.
d. Liquid nitrogen should only be stored in approved containers.
e. Do not touch any non-insulated surface cooled to liquid nitrogen temperatures, as adhesion of the skin will occur, due to freezing of the moisture layer at the interface, resulting in contact burns. Handle all cooled objects with tongs or forceps, and do this without undue delay, as these will also cool rapidly by conduction.
f. Do not allow any LN2 to touch any part of your body or become trapped in clothing near the skin.
g. Never drop a liquid nitrogen container. Damage to a container may result in over-pressurization or container failure.
h. Never attempt to stop or catch a falling container.
i. Do not leave open containers unattended.
j. Liquid nitrogen containers should be stored in cool, dry and well-ventilated areas.
k. Do not store in a cold room or other controlled environment without air supply.
l. Liquid nitrogen containers should be stored out of direct sunlight.

9. TRANSPORT OF LIQUID NITROGEN

a. There must be no refill of liquid nitrogen in the lab.
b. The vendor will go to the designated storage/use area.
c. The vendor will transport the tank to the service lift. A buddy system should be adopted where one to load and the other to receive at the destination floor.
d. The transport of the liquid nitrogen tanks are to be strictly carried out in unmanned service lifts only.
e. For laboratories located in MD1 and MD6, the key to the service lift can be obtained from the security counter located at MD6. Users will have to register their name and contact numbers for loan of the key, and to immediately return the key when the job is completed.

10. TRAINING

All staff/students using or handling cryogenic liquids must receive training which includes hazards associated with its use, care, selection and use of protective equipment and

emergency procedures. New users of liquid nitrogen should receive instruction in its use from safety lead or experienced members in the laboratory.

11. FIRST AID

In the event of the following:

Eye contact: Remove injured person from source of contamination, open eyelids and allowing liquid to evaporate. Seek medical attention and notify PI.

Skin contact:

a. For minor injuries, clothing should be loosened and the person made comfortable. Clothing should not be pulled away from burned or frozen skin.
b. Exposed tissues should be restored to normal body temperature by running warm water (40°C) over the affected part.
c. Do not rub or massage the affected parts of the body.
d. Seek medical attention immediately and notify PI.

Asphyxiation:

a. If a person is overcome by loss of oxygen while working with cryogenic liquids, the victim (who may be unconscious) should be removed to a well-ventilated area immediately.
b. The person should be kept warm and rested whilst medical attention is obtained.
c. Seek medical attention and notify PI.

12. INCIDENT REPORTING

Accidents resulting in injuries must be reported to the PI and/or laboratory safety lead immediately after first aid is applied.

Seek medical attention when necessary at the University Health Centre or proceed to the Accident & Emergency units of National University Hospital after office hours.

All incidents or accidents have to be notified to OSHE within 24 hours via the online NUS Accident and Incident Management System (AIMS) @https://inetapps.nus.edu.sg/osh/portal/eServices/ehs360_aims.html. The AIMS report can be submitted by the injured staff/student, safety leads, his or her supervisor/representative if the staff or student is unfit/unable to do the initial report.

13. REFERENCE

NUS Laboratory Chemical Safety Manual (NUS/OSHE/M/02)

14. REVISION HISTORY

Date Revised	Version No.	Author	Summary of Revisions
20-03-2016	001	Yeo Soh Bee	
01-10-2016	002	Yeo Soh Bee	Section 12: Revised Accident and Incident Reporting System (AIRS) to Accident and Incident Management System (AIMS)
15-04-2019	003	Yeo Soh Bee	New addition: Section 5: added cryogenic apron Section 7.2 Cryogenic Tubes Section 7.3 Self-Pressurizing Tanks Section 8(h): added cryogenic apron
29-10-2021	004	Adeline Chow	Update of HOD. Section 9: Update of transport of liquid nitrogen.