



Liquid Nitrogen Safety and Monitoring SOP

Background Information

Liquid Nitrogen (LN) is a colorless, odorless, extremely cold liquid and gas under pressure. Contact with liquid or cold vapors can cause severe frostbite. Cold vapors in the air will appear as a white fog due to condensation of moisture. Nitrogen gas is colorless, odorless and non-flammable. The primary health hazard is asphyxiation due to displacement of oxygen. CAP and OSHA requirements state that the laboratory must have adequate policies and procedures for the safe handling of LN.

Purpose

This Standard Operating Procedure establishes procedures for safe handling and monitoring of LN in cryogenic refrigerators and dewar flasks. This procedure applies to all laboratory personnel who will be handling LN.

Terms and Abbreviations

LN – Liquid Nitrogen

Cryogenic liquid: liquid with a normal boiling point below -130°F (-90°C). Common industrial gases transported, handled and stored in the liquid state at cryogenic temperatures are Argon, Helium, Hydrogen, Nitrogen, and Oxygen

Dewar Flask: Liquid dewar flasks are non-pressurized, vacuum-jacketed vessels, similar to a Thermos bottle. Dewars are designed with either loose-fitting caps or pressure relief valves, that prevents air and moisture from entering, yet allows excess pressure to vent

Hazards/Special Properties

- A. Frostbite/Tissue Damage - Cryogenics are extremely cold and can cause instant, severe frostbite and tissue damage. Cryogen vapors can freeze the skin or eyes faster than liquid contact, and even faster than metal contact. Direct contact with cryogenic liquids, un-insulated cryogenic pipes or equipment can cause freeze burns and tissue damage. The fluid in eyes will freeze in contact with a cryogen, causing permanent eye damage.
- B. Asphyxiation - On vaporization, cryogenic liquids will expand at least two orders of magnitude. LN, the most commonly used cryogenic liquid, will expand over 700 times. Liquid oxygen will expand almost 900 times. If a cryogenic liquid is vaporized so as to reduce the oxygen percentage below 19.5%, there is a risk of oxygen deficiency and asphyxiation. In confined space or poorly ventilated areas (such as cold rooms, elevators, or storage rooms), the expanding gas can displace oxygen, presenting an asphyxiation hazard to staff working in the area. Simple asphyxiants such as nitrogen do not have good warning properties. To prevent asphyxiation hazards, cryogenic liquids must be stored and used in well-ventilated areas.
- C. Pressure Buildup and Explosions – Because of their high liquid to gas expansion ration, cryogenic liquids present a potential explosion hazard when they evaporate. Cryogenics will boil as they sit in their storage vessels by absorbing heat energy from the warmer surroundings. The vessels used to store cryogenic liquids must have a pressure relief valve or venting lid to allow for the release of evaporated gas from the container.



- D. Oxygen Enrichment - When transferring LN through un-insulated metal pipes, the air surrounding a cryogen containment system can condense. Nitrogen, which has a lower boiling point than oxygen, will evaporate first. This can leave an oxygen-enriched condensate on the surface that can increase the flammability (combustibility) of materials near the system, creating potentially explosive conditions. In order to minimize the fire hazard potential, equipment containing cryogenic fluids must be kept clear of combustible materials.
- E. Material Brittleness – Cryogenic liquids cause many common materials such as carbon steel, plastic and rubber to become brittle or possibly fracture under stress.

General Handling Procedures

- A. Remove metal jewelry/watches on your hands and wrists before working with cryogenes. If exposed to cryogenic liquids or boil-off gases, jewelry can freeze to the skin.
- B. Wear protective clothing. Cover all exposed skin by wearing long sleeve shirts, long pants (cuff-less), a long sleeve lab coat, well-fitted leather shoes (no sneakers or sandals) and gloves. Gloves should be loose-fitting, lightweight, flexible, and insulated to allow for quick removal if cryogenic fluids are spilled on them. Wear a cryogen apron when a splash potential exists or when large quantities of cryogenes are handled.
- C. Protect your eyes by wearing safety goggles whenever working with cryogen fluids. Full face shields shall be used in the following situations: when a cryogen is poured; for open transfers; or if fluid in an open container may bubble.
- D. Handle cryogenic liquids carefully. Do not allow unprotected areas of skin touch objects cooled by cryogenic liquids. Use tongs to withdraw objects immersed in the liquid and handle the object carefully.
- E. Transfer or pour cryogenes slowly to minimize boiling, splashing and spilling. Use proper transfer equipment, such as a phase separator or special filling funnel (the top of the funnel should be partly covered to reduce splashing). If the liquid cannot be poured, use a cryogenic liquid withdrawal device for the transfer (be sure to follow all instructions provided with the device).
- F. Do not overfill containers. Do not use hollow rods or tubes as dipsticks since liquid could be released from the top of the tube. Instead, use wooden or solid metal dipsticks.
- G. Store and use cryogenics in a well-ventilated area. In closed areas, gases can reduce the oxygen concentration and can result in asphyxiation. To avoid asphyxiation, an oxygen monitor is recommended when working with a cryogen in a confined space.

Dewars

- A. Use containers specifically designed for low-temperature liquids, such as a dewar. Liquid dewar flasks are non-pressurized, vacuum-jacketed vessels, similar to a Thermos bottle. Dewars are designed with either loose-fitting caps or pressure relief valves that prevents air and moisture from entering, yet allows excess pressure to vent. Do not connect the tank and the dewar tightly to avoid pressure build up in the dewar. Do not use any stopper or other device that would interfere with venting of gas.
- B. Cryogenic containers are designed and made of materials that can withstand rapid changes and extreme temperature differences encountered in working with cryogenics. Fill containers slowly to minimize internal stresses that occur when any material is cooled.



- C. When hand-carrying cryogen-containing dewar, ensure the dewar is your only load (don't carry anything else). Watch for people who may run into you, and ensure that the dewar is carried with both hands and as far away from your face as comfortably possible.
- D. Ensure dewars are properly labeled with the identity of the cryogen. Do not mix different cryogens in the same dewar.
- E. Use care when filling portable dewars and do not overfill them.
- F. Do not cover or plug the entrance opening of any dewar. Do not use any stopper or other device that would interfere with venting of gas.
- G. Keep containers upright at all times except when pouring liquids from dewars specifically designed for that purpose. Handle containers gently; rough handling can cause serious damage to dewars and refrigerators. Dropping the container can cause partial or complete loss of vacuum. Do not walk, roll or drag these containers across a floor; uses a dolly or handcart.
- H. Keep containers clean and dry. Moisture, chemicals, strong cleaning agents may promote corrosion which should be removed promptly. Use water or mild detergent for cleaning and dry the surface thoroughly. Do not use strong alkaline or acid cleaners that could damage the finish and corrode the metal shell.

Pre-analytic Procedure

Refer to the following procedures:

- *Enter other basic procedures needed for your laboratory*

Analytic Procedure

Supplies:

- Cryogenic liquid handling gloves
- Face shield
- Oxygen monitor with alarm
- Cryogenic containers
- Dewars flasks

Equipment Calibration/Maintenance

1. Record LN level daily.
 - a. If level is <25%, initiate and document LN re-supply procedure.
2. Record LN Temperature daily
 - a. If temperature is outside acceptable ranges, notify the section supervisor.
3. Record Oxygen level daily
 - a. If level is outside acceptable ranges, notify the section supervisor and safety officer.



4. Perform weekly cleaning procedure
5. Document all procedures and issues on the Liquid Nitrogen Monitoring and Maintenance Log (see related documents).

Quality Control

- A. LN Level and temperatures must be monitored and documented daily.
- B. (List the minimum acceptable LN level to determine and initiate the re-supply process)

Test Method Instructions

1. Safety Warnings and Precautions-- The safety precautions in this SOP must be followed to avoid potential injury or damage. Do not attempt to handle LN until you read and fully understand the potential hazards, their consequences, and the related safety precautions.
2. Notify another staff member before working with LN or in a LN storage room.
3. Use cryogenic containers that are specifically designed for LN and made of materials that can withstand extreme temperature differences. These containers should be filled slowly to minimize internal stresses that occur when any material is cooled.
4. Do not cover or plug any opening on LN refrigerators or dewars flask. Do not use any stopper or other device that could interfere with the venting of gas.
5. Cryogenic liquid containers are designed to operate with minimal or no internal pressure. Inadequate venting can result in excessive pressure, which could damage or burst the container. Use only the loose-fitting neck tube core supplied or one of the approved accessories for closing the neck tube. Check the unit periodically to be sure VENTING is not restricted by accumulated ice or frost.
6. Use a phase separator or special filling funnel to prevent SPLASHING and SPILLING when transferring LN into or from a dewar flask or refrigerator. Use only small, easily handled dewars flasks for pouring liquid. For larger, heavier containers, use a cryogenic liquid withdrawal device to transfer liquid from one container to another. Be sure to follow all instructions supplied.
7. **Do Not Over Fill Containers.**
Filling above the bottom of the neck tube (or *specified maximum level*) can result in OVERFLOW and SPILLAGE upon closing.
8. Never Use Hollow Rods or Tubes as Dipsticks. When a warm tube is inserted into LN, liquid will spout from the top of the rod or tube. This can result in a risk of facial exposure due to gasification and rapid expansion of the liquid inside the tube.
9. **Nitrogen Gas Can Cause Suffocation without Warning**
Store and use only in a well-ventilated area. As liquid evaporates, nitrogen gas is released and displaces normal air. This reduces the concentration of oxygen and can result in asphyxiation.
10. Nitrogen gas is colorless, odorless and tasteless. It cannot be detected by human senses and may be breathed as if it were normal air. Breathing atmospheric air that contains less than 18% oxygen causes dizziness and quickly results in unconsciousness and death.



11. The cloudy vapor that appears when LN is exposed to air is condensed moisture; not the gas itself. The issuing LN gas is invisible.
12. Never dispose of LN in confined areas or places that may expose others to the gas. Dispose only outside and in a safe place. Pour the liquid slowly on gravel or bare earth where it can evaporate without causing damage. Do not pour liquid on pavement. Do not inhale the vapors.
13. **Protective Clothing** - Always wear protective face covering, insulated gloves and long-sleeved clothing to help prevent unnecessary exposure to LN.
14. **Contamination** - If containers stored in LN contain hazardous biological materials, they must be opened in appropriate biological safety cabinets only and in accordance with (*your lab's*) safety policy for handling potentially infectious substances. Broken containers within storage freezers pose a risk of contamination due to potential survival of the released contents. Handle all broken containers in accordance with (*your lab*) policy.
15. **Explosion** - LN is a cryogen with a boiling point of -196°C (-320°F). When removed from a LN atmosphere, improperly sealed sample containers may explode. Placing containers in vapor phase nitrogen for several hours before immersing them in LN minimizes the risk of explosion.
16. If exposure to LN causes any adverse effects, follow steps outlined below.
 - a. (List procedures according to your laboratory's safety policy)

References

1. Material Safety Data Sheet, Nitrogen, CAS Number 7727-37-9 LN.
2. College of American Pathologists (CAP) 2025. Commission on Laboratory Accreditation, Laboratory Accreditation Program; Laboratory General Checklist, Revised 12/09/2025.
3. DAIDS Good Clinical Laboratory Practice Guidelines, Version 4.1, Effective 08/16/21.
4. Occupational Safety and Health Administration. (1970). Occupational safety and health standards: Compressed gases (general requirements) and Hazard Communication. OSHA Standard No. 1910.101 and 1910.1200. United States Department of Labor.
5. CLSI. Laboratory Safety Management, First Edition, CLSI document QMS28 ED1, Wayne, PA: Clinical Laboratory Standards Institute; 2025.

Related Documents

Liquid Nitrogen Monitoring and Maintenance Log