Liquid Nitrogen Dewar

Monitoring and Maintenance

Mutant Mouse Resource and Research Center

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Purpose:

This SOP establishes procedures for the safe handling and monitoring of liquid nitrogen (LN_2) in cryogenic Dewars along with maintenance and reporting of issues concerning LN_2 Dewars located in the Cryo-storage room. This procedure applies to all laboratory personnel who will be handling LN_2 and/or using samples located in any of the MMRRC Dewars located at the cryo-storage room.

Background:

Dewars

A. Use containers specifically designed for low-temperature liquids (LN_2), 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 close 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.

Hazards/Special Properties

A. Frostbite/Tissue Damage - Cryogens 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. Liquid nitrogen, the most used cryogenic liquid, will expand over 700 times. Liquid oxygen will expand almost 900 times. If a cryogenic liquid is vaporized 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 asphyxiates 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. Cryogens 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 liquid nitrogen 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. 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.

Safety Instructions:

If at any time, you hear or see oxygen sensor alarm sounding, please leave the area immediately and contact person with information posted on the door of cryo-storage room.

General Handling Procedures

1. Remove metal jewelry/watches on your hands and wrists before working with cryogens. If exposed to cryogenic liquids or boil-off gases, jewelry can freeze to the skin.
2. 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 cryogens are handled.
3. 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.
4. 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.
5. Transfer or pour cryogens 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).

6. Do not overfill containers. Do not use hollow rods or tubes as dipsticks since liquid could be release from the top of the tube. Instead, use wooden or solid metal dipsticks.
7. 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.

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Materials:

- Cryogenic liquid handling gloves and aprons
- Face shield
- Cryogenic containers
- LN_2 measuring stick

Procedure:

1. Enter the cryo-storage room if the oxygen sensor alarm is not going off. If it is going off, please contact the person with information posted on the door of the cryo-storage room.
2. The Custom Biogenic Systems' Isothermal V-3000AB Dry Storage used to store MMRRC materials is connected to a LN2 silo provided by Linde Gas & Equipment (former Praxair) to provide auto filling. The inside temperature is monitored by a continuous automated temperature *monitoring system provided* Rees Scientific (https://www.reesscientific.com/).
3. Please check the gauge located at the end of LN_2 supplying pipe. The reading should be 20~22 PSI.
4. Please check all the display panels of all five dry Isothermal V-3000AB Dry Storages for any abnormalities.
5. If there is any issue with the LN_2 Dewar, log it in the Appendix B and contact the appropriate supervisor.
6. Supervisors: Follow up the issue and log in details steps taken to resolve the issue.
7. To retrieve sample(s), wear protective goggles and cryo apron located at the cryoware station.
8. Unlock the lock of the Dewar using a key. Lock the lid after sample retrieval.
9. For small Dewar monitoring, measure the LN_2 by LN_2 measuring stick and record the details in the Appendix C. The monitoring log is common for all the small Dewars so carefully note the details in the right column.

10. The Rees system will call sequentially for the phone numbers of emergency responders. If Rees system calls, please respond promptly to locate the Dewar, determine the problem, and fix the issue accordingly. The Isothermal V-3000AB Dry Storage can be filled with LN_2 by manually press the fill button.
11. In case of LN_2 supply issue, do not open the Dewars. A fully filed Isothermal V-3000AB Dry Storage can maintain temperature below -150°C for 72 hours without opening. Contact Linde (Praxair) or Airgas as soon as possible for LN_2 supply.

References:

1. http://resources.psmile.org/resources/facilities-safety/safety/liquid-nitrogen/Fac1.2-03%20Liquid%20Nitrogen%20.doc/at_download/file
2. 1. Forma Scientific, Inc. P.O. Box 649, Marietta, Ohio 45750 USA. TEL (740) 373-4763 Telefax (740) 373-4189. Manual No. 7007400.
3. Clinical Laboratory Standards Institute (CLSI). *Clinical Laboratory Technical Procedure Manuals; Fourth Edition*. CLSI Document GP2-A4 (ISBN 1-56238-458-9). Clinical and Laboratory Standards Institute, Wayne, PA
4. https://www1.udel.edu/ehs/research/downloads/liqnitrosopchem.pdf

Appendix A. Contact Information for Person/s in charge of liquid nitrogen monitoring and Dewar maintenance and company representatives

If there are any issues, report all problems to:

1. Yuksel Agca: agcay@missouri.edu
2. Hongsheng Men: Menho@missouri.edu
3. Linde (Praxair) representative from Columbia, MO
   Jeff DeShon
   Jeff_seshon@praxair.com
4. CHART Inc. MVE representative
   Ramon Gonzalez
   Ramon.gonzalez@chartindustries.com

Appendix B: LN_2 Monitoring and Maintenance Log

Note: Please see binder labeled: LN_2 Monitoring and Incident Reports additional copies of weekly monitoring logs and incident reports.

Dewar Name: _____

Date	Issue	Notes

Appendix C: Incident Report

Dewar Name: _____

Date	LN_2 level	Temp (°C)	Issue	Steps taken
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