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Document Contact	Wendy Frizzo: Bone and Tissue Coordinator
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Handling Liquid Nitrogen for Human Tissue Storage-Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide the Blood Bank staff with information and procedures for the safe handling of liquid nitrogen utilized for storing and transporting cardiovascular tissue.

II. CLINICAL SIGNIFICANCE:

A. Cardiovascular tissue is stored in the Blood Bank for purposes of transplantation. These grafts are stored in a cryogenic Dewar, the Isothermal V-1500 Series. The refrigerant used in this Dewar is liquid nitrogen. The liquid nitrogen is contained inside the Dewar's walls, and the stored tissue never comes into contact with the liquid nitrogen except in the case of an accidental overfill.

B. Liquid nitrogen is a cryogenic material. Cryogenic materials are those substances that must be cooled to an extremely low temperature to change from a gas to a liquid. Cryogenics are characterized by their extremely low temperature and by their extremely high expansion rate when their physical state changes from liquid to gas. Due to these characteristics, work involving liquid nitrogen presents certain health and safety hazards. Those working with liquid nitrogen need to be aware of such hazards and take all necessary precautions.

III. DEFINITIONS:

A. **Cryogenic material**: Substances that must be cooled to an extremely low temperature to change from a gas to a liquid.

B. **Inert gas**: Any gas that is non-oxidizing.

C. **Cryogen**: A liquid, such as liquid nitrogen, that boils at a temperature below - 160°C and is used to obtain very low temperatures; aka: a refrigerant.

D. **Liquid nitrogen**: Nitrogen in its liquid state.

IV. HAZARDS OF HANDLING LIQUID NITROGEN:

A. **Liquid nitrogen burns and frostbite hazards**: Liquid nitrogen is an extremely cold liquid, -196°C. Liquid nitrogen that is allowed to come in contact with human skin can cause severe damage to living tissue, including cold burns and frostbite. Damage can occur very quickly with only brief contact, longer than only a few seconds. Liquid nitrogen has a very low boiling point. The gases released can produce frostbite and permanently damage delicate tissues, i.e. eyes.

B. **Flammability**: Liquid nitrogen is associated with explosion hazards, and care must be taken to avoid such hazards. Liquid nitrogen, a liquefied inert gas, may condense oxygen from the atmosphere causing oxygen entrapment in unsuspected areas. In addition, extremely cold surfaces are also capable of condensing oxygen from the atmosphere. Liquid nitrogen storage and usage should occur in a wide open space.

C. **High-pressure gas**: Working with or storing liquid nitrogen presents hazards from high-pressure gas. Liquid nitrogen is stored at or near its boiling point. Therefore there is always some gas present in the container. Due to the large expansion ratio from liquid to gas, a build up of high pressure can occur when the liquid evaporates. The evaporation rate will depend on the fluid, storage container design and environmental conditions. The container capacity must include an allowance for the evaporation of the liquid into the gaseous state. To prevent hazards associated with high-pressure gas, it is important to ensure that pressure relief devices are used appropriately. These devices should be maintained and checked regularly for leaks or damage. Pressure relief devices must be sized for maximum possible back pressure. These checks are done by the supplier.

D. **Displacement of Oxygen/Asphyxiation**: Liquid nitrogen is capable of causing asphyxiation by displacing breathable air. To avoid such conditions, natural ventilation should be used.

V. PERSONAL PROTECTIVE EQUIPMENT:

A. Cryogenic gloves (large enough to allow quick removal)

B. Cryogenic apron and/or lab coat

C. Goggles or safety glasses

VI. SPECIAL SAFETY PRECAUTIONS:

Because of its low temperature, liquid nitrogen will cause frost bite to the skin much in the same way as hot liquids can burn. For this reason, always wear the proper protective clothing when handling liquid nitrogen. In addition to the listed personal protective equipment, it is preferable that closed toed shoes with backs (no clogs) be worn to avoid accidental spilled liquid from entering your feet. Pant legs which come down over the tops of shoes will provide further protection.

VII. PROCEDURE:

A. **Changing the liquid nitrogen supply**:

Step	Action	Notes
1	Put on a pair of cryogenic gloves, cryogenic apron or lab coat, and safety glasses or goggles.	• Exposed skin surfaces of the handler should be minimized. • Gloves should fit loosely and the handler should be able to shake the gloves free from the hands.
2	Turn the manual **liquid** valve located on the top of the liquid supply tank to the right until it can be turned no further.	• This action will close the flow of liquid nitrogen to the cryogenic vessel.
3	Disconnect the supply line from the liquid nitrogen supply tank by using the adjustable wrench to loosen the nut directly attached to the manual **liquid** valve.	• Support the T-Valve Assembly with the other hand while performing this action.
4	Attach the supply line onto the manual **liquid** valve of the liquid nitrogen supply tank labeled **FULL.**	• Tighten the nut with an adjustable wrench to ensure the hose is firmly connected to the supply tank and will not leak. • Never attach the supply hose to a manual valve labeled vent on the liquid nitrogen supply tank.
5	Turn the manual **liquid** valve to the left until it cannot be turned further.	• This action will open the flow of liquid nitrogen to the cryogenic vessel. • The storage vessel may begin to fill automatically if the liquid nitrogen level has drop below 12 inches. If the level is above 12 inches, the storage vessel will remain inactive until the lower threshold is detected.

Step	Action	Notes
6	Attach a pink label to the empty tank labeled **EMPTY.** Notify the appropriate lab assistant to order a replacement tank.	• Notify the appropriate lab assistant to order a replacement tank.

B. **Preparation of a Temporary Storage Vessel**:

1. The T-Valve assembly is used to transfer liquid into a temporary storage vessel for the purposes of transporting cardiovascular tissue to the OR. the following are key elements of the T-Valve assembly:

   a. Dewar/Storage Vessel:

Photo credit: Biolife Solutions

   b. T-Valve:

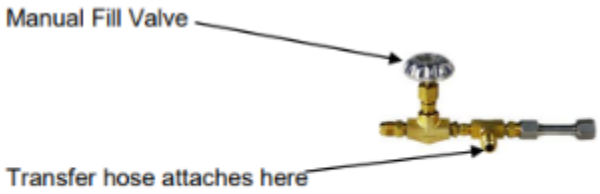
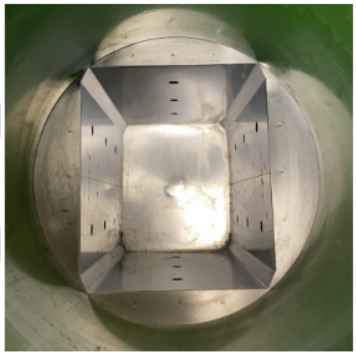


Photo Credit: Custom Biogenic Systems

   c. Liquid Nitrogen Transfer Hose:

Photo Credit: Biolife Solutions

2.

Step	Action	Notes
1	Put on a pair of cryogenic gloves, cryogenic apron or lab coat, and safety goggles or glasses.	• Exposed skin surfaces of the handler should be minimized. • Gloves should fit loosely and the handler should be able to shake the gloves free from the hands.
2	Remove the cover on the storage vessel and lower the transfer hose into the vessel. Turn the manual fill valve slowly to the **left** until liquid nitrogen begins to flow.	• Cover should be lifted straight upwards.
3	Fill the storage vessel up to the first notch from the bottom of the neck tube. Turn the manual fill valve to the **right** until it can be turned no further to stop the flow of liquid nitrogen.	 Photo Credit: William Beaumont Hospital Blood Bank Staff • The flow of liquid nitrogen should be turned off before the desired amount is reached. Liquid nitrogen continues to flow slowly after the valve is closed. • Allow the liquid nitrogen to sit until it is no longer boiling to cool the vessel.
4	Remove the transfer hose from the temporary storage vessel and allow the hose to thaw out of the way of personnel activity. Replace the cover on the storage vessel.	• Do not detach the T-Valve assembly or transfer hose from the liquid nitrogen storage tank. This assembly is meant to remain part of the entire

Step	Action	Notes
		unit.

3. Prior to transporting tissue in the storage vessel, the excess liquid should be poured off into a designated liquid nitrogen waste container.
    a. Use extreme caution when manipulating liquid nitrogen.
    b. Use all provided personal protective equipment and tools.

VIII. CLEANING AND MAINTENANCE:

Cleaning and maintenance of the Liquid Nitrogen Dewar Isothermal V-1500 Series should be performed bi-annually as follows:

Step	Task	Instructions
1	Cleaning	Use mild, non-abrasive household type cleaner for cleaning all surfaces of the unit.
2	Alarm Function Test	Follow the instructions given in the procedure, Liquid Nitrogen Alarm Function Test.
3	Liquid Level Check	Compare the reading of the LCD display to the frost line. The measurement of the frost line is performed by lowering a yard stick into the bottom of the Dewar and measuring the inner frost line. This measurement should be within ±1 inch of the LCD display reading.
4	Remote Alarm Check	Check remote alarm system to confirm connectivity to the Internet.
5	Leak Check	Check for leaks at all connection points in the liquid nitrogen lines.
6	Electrical Wire Check	Examine the backside of the Liquid Nitrogen Dewar Isothermal V-1500 Series for any loose wiring. All wires should be free of damage and plugs should be firmly in place.
7	Documentation	Document completion of tasks using form, *Cleaning and System check of Liquid Nitrogen Dewar Isothermal V-1500 Series & 2300 Controller.* A checkmark, tech initials, and date in each task indicate that the Isothermal V-1500 Series Liquid Nitrogen Dewar and 2300 Controller were checked and found to be functional. If any step of the maintenance does not fall within the indicated parameters, specify action taken to correct the issue and document on the internal variance reporting system.

IX. WARNINGS:

A. Exposure of skin or eyes: If skin or eyes come in contact with liquid nitrogen, flood the exposed

area with large quantities of unheated water. Protect frozen areas with loose, bulky, dry, and sterile dressings

B. Asphyxiation: If a person is overcome by loss of oxygen while working with liquid nitrogen, move that individual to a well-ventilated area immediately. Artificial respiration should be applied if breathing has stopped.

C. In all cases, seek medical help immediately

X. REFERENCES:

1. AABB, Standards for Blood Banks and Transfusion Services, current edition
2. American Association of Tissue Banks, Standards, current edition
3. MVE Vapor Shippers Instructions for Use, Quick Charge Technology.

Attachments

Cleaning and System Check of Liquid Nitrogen Dewar Isothermal V-1500 Series & 2300 Controller

Approval Signatures

Step Description	Approver	Date
	Ann Marie Blenc: System Med Dir, Hematopath	2/8/2023
	Kristina Davis: Staff Physician	1/26/2023
Policy and Forms Steering Committe (if needed)	Wendy Frizzo: Bone and Tissue Coordinator	12/22/2022
Policy and Forms Steering Committe (if needed)	Gail Juleff: Project Mgr Policy	12/22/2022
	Rebecca Thompson: Medical Technologist Lead	12/22/2022
	Wendy Frizzo: Bone and Tissue Coordinator	12/16/2022