STEM CELL LABORATORY (STCL)

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Liquid Nitrogen Storage System

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STCL-EQUIP-016
LIQUID NITROGEN STORAGE SYSTEM

1 PURPOSE

1.1 To describe how liquid nitrogen storage tanks (freezers) for the Stem Cell Laboratory at Duke University Medical Center are filled, maintained, quality controlled, and monitored.

2 INTRODUCTION

2.1 All cellular products, including bone marrow, peripheral blood progenitor cells, umbilical cord blood, etc. are stored in liquid nitrogen freezers at temperatures of -150°C or colder. The Stem Cell Laboratory uses a variety of liquid nitrogen (LN2) storage vessels. The majority of the freezers are liquid nitrogen storage systems with a self-contained electronic level controller with a three-sensor liquid level controller, with high and low level alarm capabilities. Vapor LN2 freezers have been purchased in recent years as they have proven to be more efficient, dependable, and consume less LN2.

2.2 Each freezer is connected via an insulated LN_2 transfer hose which is connected to a regulated liquid nitrogen bulk tank supply system which is currently filled twice a week (Tuesdays and Thursdays) by Air Gas National Welders. Sometimes portable LN2 tanks are used when needed. The STCL owns two (2) 230 liter portable tanks which are stored on-site. The STCL also has a fleet of 180 liter portable tanks which are located at Dehavens Storage & Transfer. Airgas National Welders can also provide and deliver full LN2 portable tanks when ordered, if needed for special circumstances.

2.3 The LN2 equipment is maintained and serviced by Barlow Scientific contracted by the Stem Cell Laboratory to maintain and service the equipment. Barlow Scientific staff can be reached by calling 919-245-1129; this phone # can be used to reach Barlow staff during normal business hours, after hours, and/or on weekends/holidays.

2.4 Temperatures of LN2 freezers are monitored 24 hours/day, 7 days/week, using the Rees Environmental monitoring system since implementation in April 2010. The Rees system can be accessed via a web-based computer program at any time. The Rees system is programmed to notify designated personnel when alarm conditions are activated.

2.5 LN2 levels in freezers can also be measured manually (when necessary) using a yard stick. The Building's Automation System (BAS) was the alarm system used before REES Temperature Monitoring system was put in place. Since older equipment had the BAS alarms already installed, when deemed appropriate, the BAS alarms have remained in place to monitor equipment as a backup. The BAS staff can be reached for assistance by calling 919-681-2365.

2.6 Temperature tracings from the REES Monitoring System for each freezer are printed, reviewed, and filed on a monthly basis and are available in the laboratory along with the other quality control records.

3 SCOPE AND RESPONSIBILITES

3.1 Designated Medical Technologists are responsible for performing the work as outlined in this SOP. The Medical Directors and Laboratory Manager are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1	LN2	Liquid Nitrogen
4.2	PPE	Personal Protective Equipment
4.3	LED	Light Emitting Diode
4.4	BAS	Building's Automation System
4.5	STCL	Stem Cell Laboratory
4.6	REES	Rees Monitoring System (Automated)
4.7	PBPCs	Peripheral Blood Progenitor Cells
4.8	UCB	Umbilical Cord Blood
4.9	O2	oxygen

5 MATERIALS

5.1 Yard Stick

5.2 Cryo Gloves

5.3 Goggles

5.4 Face Shield

6 EQUIPMENT

6.1 Liquid Nitrogen (LN2) bulk tank system

6.2 Liquid Nitrogen (LN2) portable tanks (when needed)

6.3 Liquid Nitrogen (LN2) freezers (vapor or liquid phase)

6.4 Insulated LN2 transfer hoses

6.5 Oxygen Depletion Alarms

7 SAFETY

7.1 Always wear the appropriate personal protective equipment (PPE) when working around liquid nitrogen to include, but not limited to, LN2-specific insulated gloves, LN2-specific insulated apron, face shield, lab coat, goggles and use **EXTREME CAUTION** to avoid splashing or direct contact with the liquid nitrogen.

7.2 If the O2 depletion alarm is sounding, do **NOT** enter the room. If the O2 reading drops below acceptable limits, the alarm will sound and air should be diverted into the space you are working via air registers in the ceiling or by industrial fan.

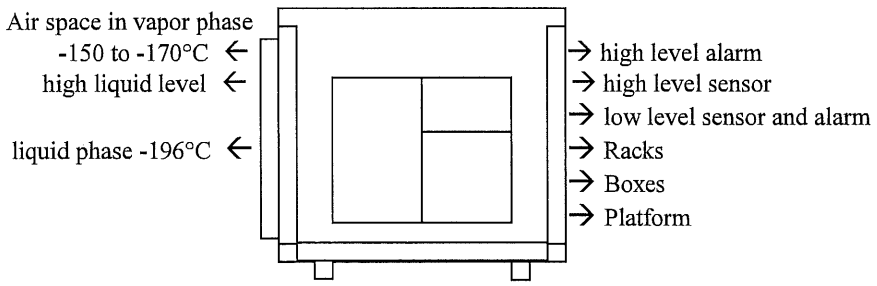
8 PROCEDURE

8.1 CONTROLLER AND ALARMS

8.1.1 Since December 1986, complete liquid immersion has been the method of choice for storing bone marrow, PBPCs and UBC in XLC series tanks as well as some other models. For immersion, an example of a specific freezer's sensor positions of the liquid level controller may be as follows for the older LN2 freezers:

High level alarm sensor	25"
High level sensor	24"
Low level sensor	20"

8.1.2 The following section view diagram illustrates the immersion system and temperatures as they exist in these older XLC-type storage tanks.



8.1.3 When the liquid nitrogen level drops below the low level sensor (less than 18 inches of liquid nitrogen) the solenoid valve will open. Liquid nitrogen enters the tank from the supply tank and fills to the high level sensor (up to the 24 inch mark) at which time the solenoid valve will close to stop the flow of LN2 into the freezer.

8.1.4 If the low level sensor is not immersed in liquid nitrogen within 10 minutes, the low alarm system is activated. The low alarm LED will illuminate and an audible alarm will sound. A mute switch can silence the alarm, but the LED will remain on as long as the condition exists. The high level alarm signals an overfill condition and is activated when the liquid nitrogen touches the high level alarm sensor (liquid level at 25 inches) for at least 2 minutes.

8.1.5 As with the low alarm, a mute switch is provided and the LED stays on until the overfill condition is corrected.

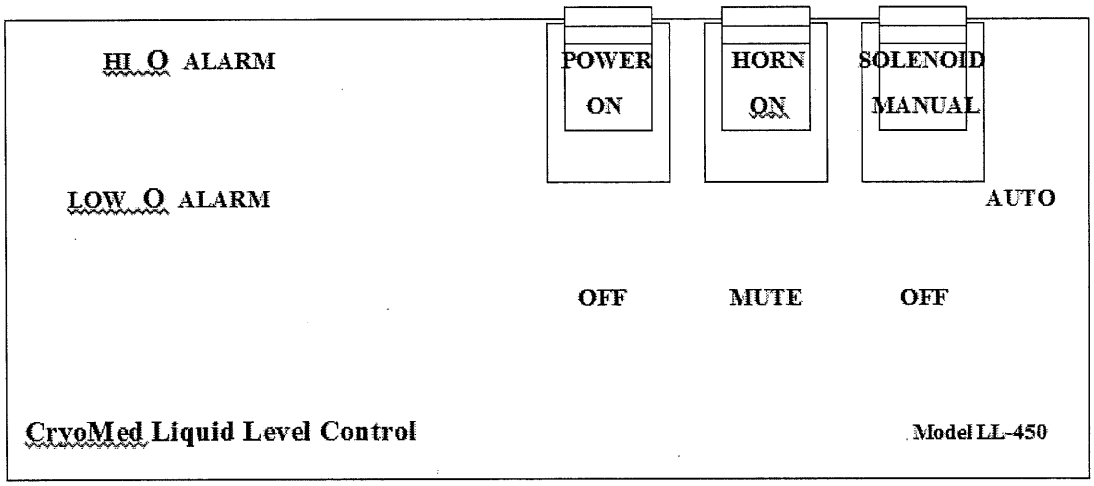
8.1.6 The alarm circuits are connected to the phone system and are monitored 24 hours a day by the Building's Automation System (BAS). In March 1989 a delay alarm system was installed for each liquid nitrogen storage tank. It consists of a small control box with a delay timer (yellow light),

an alarm (red light), and a reset button and is mounted on the wall behind each storage tank. The delay timer can be set for 0-30 minutes. When an alarm is activated it sounds to alert the Stem Cell Lab personnel and triggers the delay timer. If the alarm condition is corrected within the preset "delay" time, the alarm system returns to normal. If the alarm condition is not corrected within the time limit, the red alarm light comes on and the BAS control room is automatically alerted.

8.1.7 In April 2010, the REES Monitoring System was implemented as a method to capture data in real time 24 hours/day, 7 days/week. Since that time, there has been an overlap with the BAS system and REES. As new equipment is purchased, the BAS system is being phased out. Equipment that was purchased before 2010 will likely have both BAS and REES monitoring systems in place. Eventually the BAS will be phased out completely as the Engineering & Operations group retires that system. REES is considered the primary temperature monitoring system in the STCL since May 2012 with the BAS serving as a backup system (*when appropriate*).

8.1.8 The controller is equipped with a three position switch to operate the solenoid valve. The center position is automatic and will keep the liquid nitrogen level between the low and high level sensors. In the up position the solenoid valve is activated any time the level is below the high level sensor. The solenoid valve will remain open until the high level sensor is immerged or until the switch is placed in the off (down) position.

8.1.9 Below is a diagram of the controller panel:



8.2 NORMAL OPERATION

8.2.1 Once liquid nitrogen enters the storage tank it begins evaporating; therefore, normal operation is the process of (1) maintaining the

appropriate amount of liquid nitrogen (LN_2) in the storage tanks; and (2) keeping the supply tanks filled.

8.2.2 The freezers can automatically maintain the liquid nitrogen level between the low level sensor and the high level sensor, as previously described in section 1, as long as the controllers are functioning properly and as long as the following conditions are met:

8.2.2.1 Storage tanks are connected to 110 volt electrical outlet

8.2.2.2 Controller panel switches are set to:

Power ON (LED will light)

Horn ON

Solenoid AUTO (middle position)

8.2.2.3 Storage tanks connected via transfer hose to a liquid nitrogen supply tank with valve turned to open.

8.2.3 The liquid nitrogen levels were checked manually using the dipstick method for many years. With the implementation of the REES monitoring system in 2010, manual readings, for most pieces of equipment, were no longer deemed necessary, unless used for troubleshooting purposes, if REES was off-line, etc. The dipstick readings, when recorded manually, were often used to monitor this automatic filling operation. See section on daily quality control (section 8.3) for details regarding current practice.

8.2.3.1 The following specifications are provided by one of the manufacturers who distributes Cryomed and XLC LN2 freezers:

8.2.3.1.1 Liters of LN2/inch in storage tanks = 12 liters/inch

NOTE: The operating system of each liquid nitrogen freezer will vary as new equipment is purchased. (ie. vapor freezers etc.)

8.2.3.1.2 Static evaporation rate = 8 liters/day

8.2.3.1.3 Working daily evaporation rate = *2.8 x 8 liters/day = 22.4 liters/day

[*This factor is based upon the experience in our laboratory; the manufacturer uses a lower factor of 1.75.]

8.2.3.1.4 In our laboratory setup:

1) The LN_2 volume at high sensor = 288 liters.
2) The LN2 volume at low sensor = 240 liters.
3) The difference between the low and the high level is 48 liters; therefore, when the LN2 level drops to the low level sensor it

will open the solenoid valve and draw in 48 liters of LN2 up to the high level sensor.

8.2.4 The holding time, in days, can also be useful information to have; static and working holding times will vary depending upon how often the storage tank is entered in a 24-hour period. For practical purposes, the working holding time should be used in estimating the "worst case scenario." For example, the holding time between the low level sensor (20 inches and 4 inches), the level at which even vapor phase temperatures (another method of long term storage) can be maintained, can be calculated:

- 20 inches - 4 inches = 16 inches
- 16 inches LN_2 x 12 liters/inch = 192 liters
- Working holding time = liquid nitrogen capacity
- Working LN2 daily evaporation rate = $\frac{192 \text{ liters}}{22.4 \text{ liters/day}} = 8.6$ days

8.2.5 If no liquid nitrogen was available to replenish the LN2 storage tanks, on a daily basis, it would take 8.6 days for the liquid nitrogen to evaporate down from the low level sensor to the 4 inch mark. When the liquid nitrogen level drops below the 4 inch mark, the liquid nitrogen would need to be replenished (to maintain vapor phase temperatures) or, if LN2 is still not available, the samples would have to be relocated to an alternate storage facility or vessel. Hopefully, if the latter situation occurred, the 8.6 day buffer would allow time to arrange for alternate storage or an alternate source of LN2 from portal tanks.

8.3 DAILY QUALITY CONTROL

8.3.1 The temperatures of LN2 freezers are monitored daily using the REES Scientific Temperature Monitoring System (which tracks the temperature and alarm status of the equipment 24 hours/day, 7 days/week). As long as the REES Monitoring System is functioning properly, temperatures and/or LN2 levels will NOT be recorded on manual QC log sheets.

8.3.2 If the REES Monitoring system goes OFF-LINE (*for whatever reason*), manual QC logs will be put in place so that LN2 levels can be captured manually once/day for LN2-filled freezers or every four (4) hours for vapor LN2 freezers (*as required by regulatory standards*). Since the laboratory is not staffed on weekend and/or holidays, levels will not be monitored manually on those days. Data can be downloaded from the vapor freezers, if necessary, to obtain the readings set to be captured every four hours.

8.3.3 Manual LN2 level determinations, when deemed necessary or appropriate, can be taken using a wooden or plastic yard stick as follows:

8.3.3.1 Insert the yardstick (zero end first) along the front side of the tank. Be sure to get all the way to the bottom with the stick (the 33 inch mark should be level with the top of the tank, depending on the model of freezer).

8.3.3.2 Leave the stick immersed for approximately 5-10 seconds.

8.3.3.3 Remove the stick from the tank quickly and fan in a back and forth manner. There will be a frosted area where the stick was immersed in the liquid nitrogen.

8.3.3.4 Take the reading at the frost line. Record this value on the quality control chart designated for that freezer. Under normal operating conditions the liquid level range is 18-24 inches but the range will vary depending on the model freezer. If desired, the level in inches can be used to calculate the liters of liquid nitrogen in the tank and the holding time as described in Section 8.2.4.

NOTE: If the level is below the designated "low level" or above the designated "high level" but not in alarm, notify the Laboratory Manager or designee or the Refrigeration Maintenance Department (919-684-3232) so the problem can be resolved as quickly as possible.

8.4 SUPPLY TANK MANAGEMENT

8.4.1 To determine the level of liquid nitrogen or fullness of the 1,800 gallon bulk supply tank located at 2309B Pratt Street check the gauge reading "inches of water" on the tank, as needed for troubleshooting purposes. The tank is currently scheduled to be filled on Tuesday and Thursday evenings by Airgas National Welders. If it has been determined that a filling cycle has been skipped and a delivery is needed before the next scheduled delivery, Airgas National Welders Distribution may be contacted by calling 1-800-242-0105 to request an urgent LN2 delivery.

8.4.2 The 6,000 gallon bulk tank located just outside of the loading dock at the North Pavilion located a 2400 Pratt Street is monitored using the pressure gauge in the STCL's freezer room and via REES input # 105. The 6,000 gallon bulk tank replaced the 3,000 gallon bulk tank, that was originally in place, in late October 2015. If the gauge is registering a reading > 35 psi, call maintenance (919-684-3232, the BAS help desk (919-681-2365), etc. so the high pressure reading can be investigated immediately. If input # 105 goes into alarm, it means the level of LN2 in the bulk tank is lower than the recommended threshold; Airgas National Welders should be contacted to ensure LN2 is delivered as soon as possible.

8.4.3 The Tuesday/Friday fill schedule is subject to change during holidays, based on staffing constraints, backorder of LN2, etc.

8.4.4 The Stem Cell Laboratory also owns a fleet of portable LN2 tanks that can be used in the event that they are needed in emergent cases. The

inventory includes 230 liter tanks, 180 liter tanks, and 160 liter tanks. The 230 liter tanks are routinely stored at 2309B Pratt Street. The other portable LN2 tanks are stored off site at DeHavens Storage & Transfer.

8.4.5 Airgas National Welders can also be contacted to provide filled portable LN2 tanks when needed for unusual situations (ie. temporary equipment moves, etc).

8.5 OXYGEN DEPLETION ALARMS

8.5.1 As LN2 is filling freezers, some of the LN2 is displaced into the room. LN2 competes with O2 and can render a space oxygen-deficient if enough O2 is displaced. Since an O2-deficient environment can pose **serious health risks** to the staff working in those areas, O2 Depletion Alarms have been placed in those STCL-related areas where LN2 is used. If the O2 level in the space drops below 19.5%, the O2 depletion alarm will sound. In most cases, the sounding of this alarm triggers the HVAC system to force additional air in through the air registers at that particular location in an effort to raise the O2 level in that space. Staff should leave the room whenever the alarm sounds until the O2 level returns to normal. See the *Instructions for Oxygen Depletion Alarm* for additional details.

8.6 SPECIAL PRECAUTIONS

8.6.1 **Warning: FROST BITE** Wear cryoprotective gloves at all times when handling hoses or valves that appear frosted or when handling racks or canisters within the freezers.

8.6.2 **Warning: SPLASHING.** Wear face shield when raising or lowering racks and canisters inside liquid phase LN2 freezers. Splashing is not an issue with vapor phase LN2 freezers so face shields or goggles are not routinely needed but are available.

8.6.3 **Warning: Oxygen (O_2) Depletion Alarm.** Alarms will sound when the O2 level drops below19.5%. Leave the room immediately if the O2 depletion alarm sounds; do NOT return to the space until the O2 Depletion Alarm stops sounding and the O2 reading is >19.5%. A portable O2 meter is available for the STCL staff to use when working off-site or at locations where the operation of the O2 Depletion Alarm may be questionable.

8.6.4 **Warning: 2309 B Pratt Street Freezer Location**: STCL staff working at this location should be aware of the special precautions that must be followed when working at this location. (See *STCL-EQUIP-016 JA3 Access Instructions for 2309B Pratt Street Freezer Building* for instructions before entering this location).

8.6.4.1 Read the special instructions that are posted on the doors of the building as per Courtney Stanion, Safety and Health Specialist, Occupational Hygiene and Safety Division, Occupational and Environmental Safety Office, since November 2011.

8.6.4.2 Staff should be aware of the O2 depletion alarms that are visible outside of the building (at the main entrance) and visible and audible on the wall in the center room inside the building. If the alarms are sounding/flashing, the O2 level is below 19.5% O2 so the staff should NOT enter the building until the alarms have been cleared.

8.6.4.3 Open both doors and turn **ON** the industrial fan (the switch is on the wall inside the main entrance) to clear the air before entering the building.

8.6.4.4 Before working at this location, review *STCL-EQUIP-016 JA3 Access Instructions for 2309B Pratt Street Freezer Building* to review the signage that is currently posted on the doors at 2309B Pratt Street and the document that was distributed via e-mail back in the fall of 2011..

8.6.4.5 As an extra safety measure, STCL staff should carry the STCL's hand-held O2 meter when working at this location for extended periods of time.

The STCL's O2 meter is located on the table outside the lab manager's office in Suite 1300 of the North Pavilion at 2400 Pratt Street.

NOTE: Barlow Scientific employees routinely use their own hand-held O2 meter when working at this location.

8.6.5 **USE EXTREME CAUTION** when working with Liquid Nitrogen (LN2) or in environments where Liquid Nitrogen (LN2) is being used. Wear personal protective equipment when working with LN2 and always be aware of the O2 readings in the space in which you are working so consider your safety at all times. Cryo-gloves and face shields are available at all STCL freezer locations in which LN2 is used.

8.7 RESPONDING TO ALARMS

8.7.1 Refer to Alarm System and Instructions in the Event of Equipment Malfunction, Failure, Repair for details regarding how alarms are handled, etc.

8.7.2 Refer to Instructions for Oxygen Depletion Alarm for details regarding O2 monitor and alarms.

8.8 PREVENTATIVE MAINTENANCE of LN2 Distribution System

8.8.1 Refer to *Liquid Nitrogen Distribution System Preventative Maintenance* for details regarding preventative maintenance tasks performed, by whom, and what frequency.

8.8.2 Barlow Scientific is currently contracted by the STCL to perform quarterly maintenance on department freezers, to perform freezer validations, when requested, and to perform other freezer-related maintenance, as deemed necessary.

9 RELATED DOCUMENTS/FORMS

9.1 Alarm System and Instructions in the Event of Equipment Malfunction, Failure, Repair

9.2 Instructions for Oxygen Depletion Alarm

9.3 STCL-FORM-001 LN2 Freezer Bulk Tank Level and Pressure (as needed for manual entries)

9.4 STCL-FORM-007 Liquid LN2 Tank (as needed for manual entries of LN2 levels)

9.5 STCL-FORM-021 Vapor LN2 Tank (as needed for manual entries of LN2 levels)

9.6 STCL-EQUIP-016 FRM1 LN2 Preventative Maintenance Checklist (Barlow)

9.7 STCL-EQUIP-016 FRM2 LN2 Distribution System N. Pavilion – Inspection (Bi-Annual) Checklist (Barlow)

9.8 STCL-EQUIP-016 JA2 LN2 System – Piping Diagram North Pavilion Building Pavilion Level Piping Plan (Barlow)

9.9 STCL-EQUIP-016 JA3 Access Instructions for 2309B Pratt Street Freezer Building

10 REFERENCES

10.1 Technical Manual for the CMS-328 with LL-450 Liquid Nitrogen Storage System. CryoMed, New Baltimore, Michigan.

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
08	B. Waters-Pick	• Section 2.3 delete "the Refrigeration Department at Duke University Medical Center and/or by" and replace with Barlow Scientific and added phone #. • Section 2.5 added phone # for BAS Room. • Section 8.4.2 Change 3,000 to 6,000 gallons, date in which tank was replaced, and added phone number for BAS. • Section 8.4.3 Change fill schedule changed from Tuesday/Thursday to fill schedule of Tuesday/Friday. • Added form numbers to documents listed in section 9.

Signature Manifest

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STCL-EQUIP-016 Liquid Nitrogen Storage System

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