*If you have questions, please call Ken at any time, day or night.*

*(859) 797-0843*

1. **PURPOSE:** This SOP explains the necessary steps for maintaining human cardiac samples in liquid nitrogen dewars.
2. **SCOPE and RESPONSIBILITIES:**   
   1. Scope:
      1. The activities described in this SOP are to ensure efficient and safe storage of human cardiac samples in liquid nitrogen dewars. Weekly maintenance is required for all dewars containing specimens.
      2. Approval for samples are based on IRB #08-0338-F2L, PI: Kenneth Campbell.
   2. Responsibilities:
      1. Principal Investigator
      2. Program Coordinator
      3. Program Fellows
      4. Postdoctoral Researchers
      5. Graduate Students
      6. Undergraduate Students
3. **DEFINITIONS and ABBREVIATIONS:**
   1. Definitions:

* Biohazard - a risk to human health or the environment arising from biological work, especially with microorganisms.
* Hashcode – used to identify sample without providing any patient information.
* Liquid nitrogen – nitrogen in a liquid state at an extremely low temperature used as a coolant.
* Procurement - the action of obtaining or procuring something.
  1. Abbreviations:
* IRB – Institutional review board
* LN2 – Liquid nitrogen
* OSHA – Occupational Safety and Health Administration

1. **SAFETY AND QUALITY CONTROL:**
   1. Follow appropriate precautions based upon OSHA guidelines and/or the institutional procedure manual for the handling of biohazardous material and liquid nitrogen.
   2. The Hep B vaccine is optional but highly recommended when working with human tissue.
   3. Ensure all necessary containers are labeled with a biohazard sticker.
   4. Always keep human tissue samples on ice, dry ice, or in liquid nitrogen for proper preservation.
2. **MATERIALS, REAGENTS, and EQUIPMENT:**

Various materials, reagents, and equipment are required for the storage of human cardiac tissue samples. Generalized items may include, but are not limited to,

* Exam gloves
* Yard Stick
* Liquid nitrogen
* Liquid nitrogen dewars (R2, D2, C3)
* Cryrogenic box
* Cryogenic vials
* Paper towels

1. **PROCEDURE:**
   1. To gain appropriate access to be a part of the Procurement Team, contact the Biorepository Program Coordinator.
   2. Liquid nitrogen tank:
      1. There is a gauge on the tank. If it is 1/3 or less, a new tank should be ordered. Do not wait until the tank is empty to order a new tank.
      2. Liquid nitrogen is ordered through Scott Gross (859-252-7667). Account number UK611. Order a 165L low pressure 22psi tank to be delivered to MN508. This can take up to 2-3 days.
      3. If no one will be in the lab the next morning, leave the door unlocked so they can deliver the tank.
      4. A pink delivery ticket will be left when take is delivered. Place this ticket on the packing slip shelf.
   3. Liquid nitrogen dewars:
      1. There are 3 dewars named R2, D2, and C3.

* Model name: Locator 6 Plus
* Company: Thermo Scientific
* Catalog #CY509109CN with monitor
* Model #8209
  + 1. The dewars store the tissue in vapor phase of liquid nitrogen which is approximately - 150 °C. It is imperative the dewars are monitored to ensure samples are preserved properly.
    2. Dewar LN2 levels should be check weekly!
  1. Checking LN2 levels in dewars:
     1. There are two tiny keys in the drawer under the Banff computer station. These will unlock the dewars.
     2. Unlock dewars and remove the lid.
     3. Wear thick gloves found in glove drawer 9B (liquid nitrogen is ~- 196 °C and the vapor phase of liquid nitrogen is ~- 150 °C=very cold!!! )
     4. In the bottom of the dewer there is a hole which is ~2 inches in diameter. The depth measurement must be taken at the bottom of that hole to be accurate.
     5. Place the wooden yardstick inside the dewer with the 1 cm side of the yardstick going in first.
     6. Keep the yard stick centered so that it goes directly into the hole in the floor of the dewer.
     7. You will hear the yard stick sizzle. Give it about 20 seconds and then remove it from the dewer.
     8. Wave the yardstick a few times in the air till you start seeing frosty white glaze on it. The line where it stops being frosty tells you what the level is.
  2. Filling dewars with LN2:
     1. The maximum you should fill up the dewer is 14 cm.
     2. The minimum you should fill up the dewer is 10 cm.
     3. Place the liquid nitrogen dispenser attached to the liquid nitrogen tank inside the dewer.
     4. Then turn the valve on all the way. You know which valve to turn on by tracing back to where the dispenser is attached to the tank.
     5. Read the “open” and “close” sign direction on the value to open the valve all the way.
     6. The dispenser will make a loud noise and you will see something that looks like smoke. This is vapor phase of liquid nitorgen (don’t be afraid).
     7. Liquid nitrogen will start to dispense.
     8. You will have to wait a few minutes for the dewers to fill up.
     9. It normally fills at ~1 cm/3 min.
     10. Turn the valve off and then check liquid nitrogen level with the yardstick again.
     11. If the level is not at 14cm range, please repeat steps 6.5.3 – 6.5.10.
  3. If the dewars are not working properly contact Ken immediately.

1. **REFERENCES:**
   1. University of Kentucky Cardiovascular Biorepository IRB approved consent form
   2. UK Biosafety Manual (4.0-University of Kentucky Biological Safety Requirements, 5.3-Exposure Incidents, 10.0-Decontamination and Disposal of Biohazardous Waste)