Title: Procedure for Snap Freezing Clinical Research Samples

Purpose: To assist the laboratorian in safely freezing clinical research samples using liquid nitrogen.

Procedure:
Note: Glass tubes were not tested and are not recommended for use in this procedure. Also a Material Safety Data Sheet for liquid nitrogen is available in the MSDS Notebook located in Room A124A.

1. Observe Universal Precautions, as described in the Procedure for Universal Precautions. Wear a lab coat which is available on a rack in Room A122, thermal (blue) gloves, and use a face shield when decanting liquid nitrogen or opening the freezing container. All exposed skin should be shielded.

2. Check the gauge on top of the liquid Nitrogen tank to make sure there is liquid Nitrogen available. Should the tank be empty, and another tank is readily available, detach the hose with a wrench and attach it to the new tank. Make sure to attach it to the handle that has the ‘liquid’ ring tag and not the ‘use’ or ‘vent’ ring tag. If no other tank is available, refer to the Procedure to Order a Liquid Nitrogen Tank.

3. Liquid Nitrogen containers should be stored and used in well-ventilated areas only.

4. Place the lid of the stainless steel freezing container slightly ajar so that the hose attached to the liquid Nitrogen tank can be placed inside the container and splashing will be minimized.

5. Put on a pair of the blue thermally -insulated gloves; they may be found in Room A122 or A124. Firmly grasp and continue to hang on to the hose that is attached to the tank and place the end of the hose inside the stainless steel freezing container. Turn on the liquid nitrogen flow by turning the handle on top of liquid nitrogen tank all the way to the direction following the open arrow.

6. If freezing polypropylene simport tubes/ micro tubes, let the liquid nitrogen run for 3 minutes. If freezing mixed batches of tube types, including 12x75 mm polypropylene tubes and 5 mL Dot polypropylene tubes, let the liquid nitrogen run for 4 minutes.

7. Turn liquid nitrogen flow off by turning the handle on top of the liquid Nitrogen tank all the way to the direction following the close arrow. Place the hose on the tank’s upper ring and let it drape down. Close the lid.

8. Remove up to 4 test tubes racks from the Kelvinator and place the test tube racks on a stainless steel cart lined with a blue pad. Freeze tubes in the following order: 5 mL Dot tubes, 12x75 mm tubes and finally 2 mL simport tubes or smaller micro tubes.
Do not freeze the 2 mL simport tubes at the same time as the other size tubes; 2 mL simport tubes require less volume of liquid nitrogen. Liquid Nitrogen will evaporate while freezing, and the 2 mL simport tubes may be safely frozen when the liquid nitrogen is at a lower volume.

9. Using the blue thermally-insulated gloves, place a test tube rack of samples in the stainless steel freezing container and then place a weight on top of the test tube rack so that it will not tip. Repeat for the additional racks. Weights may be found on the shelf underneath the freezing container or at the stainless steel sink.

10. Firmly close the freezing container and wait a few minutes. Thermally-insulated gloves may be taken off during this time.

11. Again put on the thermally-insulated gloves and open the lid to the freezing container. Remove the weight on top of the rack and place on either the stainless steel cart lined with a blue pad or on a blue pad at a Sample Preparation Station. Remove the test tube rack containing the samples and place the rack again either on the stainless steel cart lined with a blue pad or on a blue pad at Sample Preparation Station. Repeat the step until the freezing container has been emptied of weights and racks. Immediately close the lid to the freezing container.

12. Check a number of random tubes from each rack to determine if they have been completely frozen. If any tubes from a test tube rack have not completely frozen, return the test tube rack to the freezing container until the samples are completely frozen. Remember to use the weights. While the remaining samples are in the freezing container, place the frozen samples in their designated storage location, according to the procedure for Sample Return to Designated Storage Location.

13. Liquid nitrogen may be reused as long as it remains in the freezing container. To freeze other samples repeat steps 6 through 12. It may be necessary to add more liquid nitrogen at this time.

14. Return all equipment to its designated area.