Risk Assessment Form

Procedure	Decanting of Liquid Nitrogen (N_2)

Name(s) of person performing the work	Users (Lab manager & Lab Technician & Tenants & Licensee's)		
Name & position of assessor	Khwaja Islam & Laboratory Manager	**Signature**	
Date of assessment	01/10/2018	**RA Number**	BioE 0032

Outline of procedure / activity:

Cryopreservation is the most effective way to preserve biological materials with minimal ageing and snap freeze tissues. The method involves the freezing and storage at very low temperatures, down to -196°C (liquid nitrogen). The critical temperature at which biological activity ceases, -147°C. The boiling point of LN2 is -196°C, relative density of gas is 0.97 (air=1) but the gas/vapour heavier than air. May accumulate in confined spaces, particular at or below low ground level.

Properties of Liquid Nitrogen:

- A colourless, odourless liquid
- Extremely cold
- Boiling point is -196°C
- Nitrogen gas is evolved which is neither toxic nor harmful
- Small volumes vaporize to give large volumes of gas (1litre gives 0.7 m3 of gas) which will displace oxygen in air. 1 litre of liquid nitrogen produces 683 litres of gas.

Known or Expected Hazards:

a) Temperature Related:

- The extremely low temperature of liquid nitrogen can cause severe burn-like damage to the skin either by contact with the fluid, surfaces cooled by the fluid or evolving gases. The hazard level is comparable to that of handling boiling water.
- The low temperature of the vapour can cause damage to softer tissues e.g. eyes and lungs but may not affect the skin during short exposure.
- Skin can freeze and adhere to liquid nitrogen cooled surfaces causing tearing on removal.
- Soft materials e.g. rubber and plastics become brittle when cooled by liquid nitrogen and may shatter unexpectedly.
- Liquid oxygen may condense in containers of liquid nitrogen or vessels cooled by liquid nitrogen. This can be extremely hazardous because of the pressure rise on the slightest degree of warming above the boiling point of oxygen (-183°C) and the possibility of explosive reaction with oxidisable material.
- Thermal stress damage can be caused to containers because of large, rapid changes of temperature.

b) Vapour Related:
- Large volumes of nitrogen gas are evolved from small volumes of liquid nitrogen (1 litre of liquid giving 0.7 m3 of vapour) and this can easily replace normal air in poorly ventilated areas leading to the danger of asphyxiation. It should be noted that oxygen normally constitutes 21% of air.
- Atmospheres containing less than 10% oxygen can result in brain damage and death (the gasping reflex is triggered by excess carbon dioxide and not by shortage of oxygen), levels of 18% or less are dangerous and entry into regions with levels less than 20% is not recommended. (Notices are posted on the freezer room lab door alerting lab workers to the dangers in case of Oxygen deficiency alarm sounding).
- Oxygen condensed into leaking containers can explode on heating following resealing or blockage with ice.

Liquid nitrogen supply tank (240 L) is stored on Level 1 freezer room (696.20.23) where 10 air exchange per hour all the time. The procedure for decanting of liquid nitrogen from supply tank (240 L) as follows:

1. Two people are required for the procedure (one as observer to help if necessary).

2. Wear appropriate PPE (lab coat, safety glasses, cryogenic apron, and Cryogloves wrist length and face shield).

3. Lab coat must be fully fastened to the top and there should be no loose items in the top small pocket of the lab coat (please remove any loose item in the top small pocket of the lab coat before decanting).

4. Use a cryogenically approved canister – pre-cool to minimize splash-back. Check canister is in good condition, undamaged neck, insulating dust/moisture cap present/in place.

5.Carry out supply tank (240 L) checks:
- regular in-service examination received and within test; appropriate location; stable & secure; check for damage & in appropriate ice formation;
- correct labelled for gas type (Nitrogen); transfer hose & filling equipment clean & free from damage;
- fit phase separator where necessary (defective vessels & hose should be removed from service & reported to maintenance);
- Check liquid nitrogen level is sufficient; check pressure gauge reading is 1 to 1.5 bar,; if above 1.5 bar consider venting down gas pressure. If pressure is insufficient to decant consider opening pressure build circuit.

6. Use the dedicated flexible stainless steel transfer hose and phase separator to prevent splashes and fogging for dispensing. Attach the transfer hose containing the phase separator to the supply tank (**blue** valve - labelled Liquid Fill/Decant) using the adjustable spanner provided. Be careful when attaching and tightening the hose that you do not miss thread and overtighten.

7. Filling Procedure:
- To decant liquid nitrogen from the supply tank (240 L) into a receiving Dewar flask (1 L or 2 L) it's a good practice to place the receiving Dewar flask in a secondary container (e.g. polystyrene box for stability and to contain any spillage);
- Place the end of transfer hose containing the phase separator into the open neck of the receiving Dewar flask (make sure to hold the hose in place for the duration of the fill).

- Open (anti-clock wise) the **blue** fill valve (labelled Liquid Fill/Decant) slightly and purge air from the hose for a few seconds then feed the transfer hose further into the Dewar.
- Control the decant allowing sufficient time for the Dewar to cool.
- Minimise mists and vapours through the control of the fill valve.
- Avoid inhaling mist and vapour clouds where possible.
- Adding liquid nitrogen to a warm Dewar flask may cause splashing and generate a significant amount of nitrogen gas. Do not leave receiving Dewar flask unattended when filling.
- When decanting the liquid nitrogen keep your head clear of heavy volume of vapour produced. Do not breathe in the vapour.
- When the receiving Dewar is full, close the fill valve on the supply tank (240 L) clockwise.
- Slowly remove the transfer hose and allow residual liquid and gas within the hose to vent off.
- Store hose correctly on supply tank.
- Make sure to close the dust/moisture cap on the receiving Dewar flask when transporting back to the laboratory.

8. Keep the receiving Dewar upright, never overfill as spillage will damage flooring and may cause injury, spill and splashes can set off oxygen depletion alarm, make sure to move receiving Dewar flask away from the sensor in the room before decanting and take care to minimise splashing as suggested will avoid alarm being set off unnecessarily.

9. Excess liquid nitrogen should be allowed to evaporate naturally inside a fume cupboard or well-ventilated area. The receiving Dewar should be left with the insulating dust/moisture cap closed.
Never pour down excess liquid nitrogen down the drain i.e. down the sink.

10. When transporting receiving Dewar's should wear PPE (lab coats, face mask, safety glasses, cryogenic apron and cryogenic gloves.

<u>Safety precautions:</u>

- Asphyxiant in high concentration.
- May cause frostbite.
- Wear suitable protective clothing.
- Keep container in well ventilated place.
- The hazard of asphyxiation is often overlooked and must be stressed during operator training.
- Before using the product in any new process or experiment, a thorough material compatibility and safety study should be carried out.
- Never leave a filling vessel unattended unless you feel your life is at risk.

Only trained personnel are allowed to decant Liquid Nitrogen.

NEVER ATTEMPT TO DECANT LIQUID NITROGEN WITHOUT ANOTHER PERSON PRESENT.

Potential hazards

Substance or item handled	Associated Hazard (s)	Existing Control Measures	Risk (L/M/H)	Further Action required	Risk (L/M/H)
Refrigerated Liquefied Nitrogen	Asphyxiation Cold burns or injury Frost bites Effects on lungs Hypothermia H280-contains gas under pressure; may explode if heated. H281-contains refrigerated gas; may cause cryogenic burns or injury.	Ensure full PPE is worn. Appropriate warning signs on the lab door. Face shield (Standard BS EN 166 39 B) and Safety glasses (Standard EN 166) worn when decanting liquid nitrogen. Cryogenic gloves (Standard EN 511) worn when decanting liquid nitrogen. Suitable footwear worn (open toed shoes must never be worn) when decanting liquid nitrogen. Consider footwear that extends up to the ankle allowing trousers to fit over the footwear. Avoid trousers with turn-ups in which spilled cryogen could collect. Guideline: ISO 20345 PPE – Safety footwear.	M	No further action required if the existing control measures are adhere to.	M

		Wear cryogenic apron (Standard EN 340) for splash & vapour protection when decanting liquid nitrogen. Suitable protective clothing worn when decanting liquid nitrogen (fully fastened Howie lab coat). Cryogenic phase separators used to prevent splashes and fogging. Only trained personnel are allowed to decant liquid nitrogen. Oxygen depletion alarm will sound and beacon will flash at a warning level when oxygen level drops below 18%. Warning personnel not to enter the room. Low level extraction in the room (30 air change per hour).			

Persons potentially at risk:

Only the user or others near by

Action in event of an accident or emergency:

1. **First Aid Measure**:
   Inhalation – In high concentrations may cause asphyxiation. Symptoms may include loss of mobility/consciousness. Remove victim to a well-ventilated area. Rescuers should not put themselves at risk; contaminated area should not be entered unless considered safe. Breathing apparatus may be required but should only be used by trained personnel. The person should be kept warm and rested, whilst medical attention is obtained. If breathing has stopped then resuscitation should be commenced by trained first aider.
   Eye contact – Rinse the eye with water immediately. Remove contact lenses, if present and easy to do. Continue rinsing. Flush thoroughly with water for at least 15 minutes. Get immediate medical assistance. If medical assistance is not immediately available, flush an additional 15 minutes.
   Skin contact – Contact with evaporating liquid may cause frostbite or freezing of skin. If clothing is saturated with the liquid and adhering to the skin then the area should be thawed with lukewarm water prior to removing the clothing.
   Ingestion - Obtain medical attention immediately.
   Emergency procedure for a major spillage or leakage – Evacuate the area and call for help (out of hours 07753 613855). Treat any individuals as above for skin, eye, and inhalation and ingestion exposure. If there is any uncertainty, then contact BOC (Tel. 0800 222 888). Restrict gas escape by closing doors behind you. Call the emergency services. Do not attempt to enter a gas-filled room without specialist training in breathing apparatus rescue techniques. Prevent people entering mists, vapours & clouds.

2. **After Any spillage:**
   - Check floors & equipment for embrittlement & other damage caused by extreme low temperatures.
   - Consider using oxygen monitoring equipment to check oxygen levels before declaring the area safe.

Arrangements for monitoring effectiveness of control:

Weekly check of tank and connections for leaks.

Annual inspection of the Liquid Nitrogen Supply Tank (240 L) by BOC.

Six monthly inspection of oxygen depletion sensors by Pollution Monitors.

Review of the Risk Assessment:

Date of review		Name of reviewer	
Date of next review		Signature	

Have the control measures been effective in controlling the risk?

Yes	No

Have there been any changes in the procedure or in the information available which affect the estimated level of risk from the listed substances

Yes	No

What changes to the control measures are required?

Receipt of Risk Assessment:

This assessment has been issued to and read by:

Name	Date of receipt	Signature
