

Risk Assessment Form

Procedure	Retrieval and storage of cryovials in cryogenic biostorage tank (Biosystem Archive 40)
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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	06/09/2018	RA Number	BioE 0033

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.

Long term storage of cryovials (cell) are stored in liquid nitrogen (vapour phase) in cryogenic biostorage tank in Level 1 freezer room (696.20.23). The Biosystem archive 40 stores 44,200 x 2 ml vials in vapour phase, has rotating drum that is sealed from LN₂ (Sample chamber sealed to keep samples clear of liquid nitrogen), reservoir of 180 litres of LN₂, external turning wheel to help locate samples (without getting hands cold), fold down steps for easy access, automatic de-fogging to help see sample racks and save LN₂, vessels fills every 3 days # saving on LN₂, twin fill solenoids as standard for extra overflow protection, access for external temperature probe, Fold away integral steps for easy access, Offset neck for low evaporation rate, Automatic quick temperature recovery, lockable lid with key.

The procedure for storage and retrieval of cryovials in liquid nitrogen (cryogenic storage tank) as follows:

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

2. Storage and Retrieval of cryovials in cryogenic storage tank:

a) Before opening the cryogenic storage tank lid, decide which racking stack you need to store your cryovials by checking with the liquid nitrogen cell storage unit stack allocation sheet, which can be found on front of the cryogenic storage tank control panel. Each racking is numbered (1-34). To reach the cryogenic storage tank lid, open up the folding integral steps, which are attached to the front of the cryogenic storage tank.

b) PPE must be worn all the time. 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 retrieval and storage of cryovials). Wear cryogenic apron over the lab coat for splash & vapour protection.

c) Open the lid of the cryogenic storage tank and using the removable turntable drive handle (found on the top of the cryogenic storage tank) to locate the rack and then pull out the rack using the handle very slowly and place the rack carefully on the cryogenic resistant floor - silver. Never place the rack on the rim of the cryogenic storage tank neck as it will damage the inner black sealant tubing and chance of drooping sample vials into the tank. Close the lid on the cryogenic storage tank immediately.

d) Remove the long thin bar from the middle of the racking stack and then take out cryovials storage box to store your cryovials in.

e) Place the cryovials storage box in the racking stack and then carefully insert the long thin bar in the middle of the racking stack (this keeps the cryovials storage box in place so they won't fall out from the

racking stack).

f) Open the lid of the cryogenic storage tank and carefully and slowly insert the rack using the handle into the cryogenic storage tank. Close the lid on the cryogenic storage tank immediately. Place the removable turntable drive handle back on top of cryogenic storage tank and fold the integrated steps.

Only trained personnel are allowed to retrieve and store cryovials in liquid nitrogen in cryogenic storage tank. 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)
Liquid Nitrogen	<p>Asphyxiation</p> <p>Cold burns or injury</p> <p>Frost bites</p> <p>H280-contains gas under pressure; may explode if heated.</p> <p>H281-contains refrigerated gas; may cause cryogenic burns or injury.</p> <p>Explosion due to trapped, expanding gas</p>	<p>Container kept in well ventilated room with low level extraction in case of spillage. This will be triggered when the oxygen alarm is triggered below 18%. There is 6 air exchange per hour in the room all the time.</p> <p>Wear appropriate PPE.</p> <p>Face shield and Safety glasses (Standard EN 166) worn.</p> <p>Wear cryogenic apron (Standard EN 340) for splash & vapour protection.</p> <p>Cryogenic (Standard EN 511) gloves worn.</p> <p>Suitable footwear worn (open toed shoes must never be worn). Guideline: ISO 20345 PPE – Safety footwear.</p>	M	No further action required if the existing control measures are adhere to.	M

		<p>Suitable protective clothing is worn (fully fastened Howie lab coat).</p> <p>When thawing cryovials, secondary container (e.g. polystyrene box) is used to reduce explosion risk.</p> <p>Only trained personnel are allowed to retrieve and store cryovials in cryogenic storage tank.</p> <p>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.</p> <p>Low level extraction in the room (30 air change per hour).</p>			
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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).

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
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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
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What changes to the control measures are required?

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Receipt of Risk Assessment:

This assessment has been issued to and read by:

Name	Date of receipt	Signature

