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Title: Use of NDCLS Cryostorage Facility	
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DSO sign off (name): Dr Andrew Graham 	**Date SOP issued**: 09 December 2016
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LOCAL DETAILS			
Division	Nuffield Division of Clinical Laboratory Sciences	**Department**	Radcliffe Department of Medicine
Area of application (building & rooms)	Room 4A10B, Level 4 Academic Block, John Radcliffe Hospital		

REASSESSMENT HISTORY (to record annual re-assessments without changes to content & version number)			
Date	**Name of assessor**	**Date**	**Name of assessor**
1.		**4.**	
2.		**5.**	
3.		**6.**	

REVIEW HISTORY (to record changes to contents)			
Date	**Details of review**	**Version number**	**Name of reviewer**
1.			
2.			
3.			

YOU ARE INSTRUCTED TO READ THE FOLLOWING THOROUGHLY BEFORE PROCEEDING TO UNDERTAKE THE METHODS DESCRIBED.

UNDER NO CIRCUMSTANCES ARE THESE INSTRUCTIONS TO BE AMENDED OR ALTERED IN ANY WAY OTHER THAN BY THE AUTHOR, APPROVER OR AN APPROPRIATE ALTERNATIVE.

This Standard Operating Procedure (SOP) document is issued by the Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine. Health and safety inspectors seek to secure compliance with the law and may refer to this document as illustrating good practice. All staff using the NDCLS Cryostorage Facility should adopt the measures set out within this document to ensure that their health, safety and welfare are not knowingly compromised. This document relates to all users, who have a common responsibility to use the facilities correctly and report any failing in the facility, or its associated equipment, to the Cryostorage Facility Manager (or in his absence Demin Li), or the NDCLS Facilities Manager Andrew Graham.

1.1 General Information regarding Handling of Liquid Nitrogen (LN_2)

Liquid Nitrogen (LN_2) poses three major, potentially fatal, hazards:

1) **Asphyxiation** by reducing available breathable oxygen. Nitrogen gas is odourless, colourless and tasteless. When LN_2 evaporates it reduces the oxygen concentration in the air and may act as an asphyxiant in confined spaces. A person can become unconscious without any warning symptoms. If the oxygen content is reduced by only a few percent from normal, there can be a substantial risk to life. Even reduced levels of oxygen, which are not immediately life threatening, can affect behaviour and judgement thereby inhibiting decision making (1).

2) **Severe burns** if the appropriate personal protective equipment (PPE) is not worn. LN_2 has a boiling temperature of -196°C at atmospheric pressure. Direct contact can freeze the skin causing frostbite and cold burns. Brief exposure to cold gas alone, whilst not affecting skin, can damage delicate tissue such as eyes, and can cause breathing difficulties in people with lung conditions e.g. asthma. .

3) **Physical trauma due to sudden pressure release** if the appropriate personal protective equipment is not worn. LN_2 has a liquid to gas expansion rate of 1:694 at 20°C. This means that, as it vaporizes, the volume it occupies will expand close to 700 times. If LN_2 gets into a vial, this expansion rate is what can causes vials to explode when removed from LN_2 storage. LN_2 can get into a vial if the vial is defective or past its expiration date, if the vial is over- or under-tightened, if there is water on the vial threads, or if the wrong types of vials are used.

There are additional hazards associated with using LN_2 storage containers in this room, specifically

1) **Risk of injury from incorrect manual handling and/or working at height** since a full tower can be heavy and a Kik-stool may be required to reach and remove it from the storage vessel.

2) **Rapid unconsciousness** will also pose a risk of falling, possibly from height

To minimise risk, all users **must** ensure that at least one other person is aware of them using the room and is able to provide assistance. **During weekends, evenings and early mornings, when numbers of staff within the department are reduced, it is essential that at least two members of staff are present in or immediately near the LN_2 facility whilst work takes place**.

1.2 Specific information regarding the NDCLS Cryostorage Facility (room 4A10B)

1.2.1 Emergency contact numbers

The nearest telephones are located inside Room 4A10B, in Lab 4A10A (Adjacent to Cryostorage Facility) or in room 4A13 (opposite Cryostorage Facility).

1) **Hospital Switchboard 0**
2) **Cryostorage Facility Manager Duncan Gascoyne 07963 800962**
3) **Demin Li 07889 225794**
4) **John Radcliffe Hospital Estates 20600**
5) **John Radcliffe Hospital Security 57727**

1.2.2 Additional responsibilities

1) **Facility Manager (Duncan Gascoyne):** ensuring at least annual review of this document
2) **Banham Group Lab Safety representative (Massimo Masiero)** for ensuring monthly checks on safety equipment and PPE
3) **NDCLS Facilities Manager (Andrew Graham)** for ensuring that yearly maintenance checks (according to Level 1 service contract) of oxygen sensor equipment and extraction fan (by Lab Mode Ltd, Elstree 01908 768000, office@labmode.co.uk) are carried out

1.3 References for this SOP document

1.3.1. HSE Information Sheet 'The risks posed by exposure to inerting gases in the open air' Offshore Information Sheet 4/2008
http://www.hse.gov.uk/offshore/infosheets/is4-2008.pdf

1.3.2. Oxygen Monitoring System manuals
http://www.quantumcryogenics.com/overview/gas-monitoring-systems/control-panel-and-repeaters/cryopanel-4.htm
http://www.quantumcryogenics.com/overview/gas-monitoring-systems/oxygen-monitoring/qfm330.htm

1.4 Related documents for this SOP

1.4.1 UNIVERSITY POLICY STATEMENT S4/03 LIQUID NITROGEN

1.4.2 UNIVERSITY POLICY STATEMENT S3/11 WORK AT HEIGHT

1.4.3 UNIVERSITY POLICY STATEMENT S8/10 EYE PROTECTION

1.4.4 UNIVERSITY POLICY STATEMENT S3/02 PERSONAL PROTECTIVE EQUIPMENT

1.4.5 UNIVERSITY POLICY STATEMENT S7/99 MANUAL HANDLING OPERATIONS REGULATIONS 1992

1.4.6 UNIVERSITY SAFETY OFFICE MEMO M23/08 EXPLOSION RISK – SAMPLE RETRIEVAL FROM CRYOGENIC STORAGE

1.4.7 NDCLS RISK ASSESSMENT DOCUMENT NDCLS-RA-001 SAFE USE OF CRYOGENIC LIQUIDS

2 Procedures

2.1 Emergency procedures

Emergencies include but are not limited to:

- any escape of liquid nitrogen that cannot be controlled including explosion of a storage vessel
- spillage of more than 50ml liquid nitrogen generating significant volume of nitrogen gas
- debilitating physical injury occurring whilst in the room (such as becoming trapped by any of the equipment or accidental self-injury)
- exposure of personnel to liquid nitrogen resulting in significant burns;
- failure of the extraction system
- fire alarm sounding

- discovery of an unconscious person

Audible alarms and/or flashing lights will signify all of these conditions apart from personal physical injury. If alarms are sounding and/or lights are flashing **DO NOT ENTER THE ROOM.**

In an emergency situation

1.1.1. Leave the Cryostorage Facility room by pressing the exit button (see picture) or through the open door. Switch on the fan then close the door.
1.1.2. Instruct anyone else in the room to leave.
1.1.3. Prevent other people from entering the room.
1.1.4. Inform another member of staff to assist if necessary
1.1.5. In most cases leave the door open, however in the case of a LN_2 spill/leak, Summon help from a first aider if required for example if there is a cryogenic burn. **All accidents/ incidents/near misses must be reported** in line with NDCLS procedures in order to improve future safety of the Cryostorage Facility. See also Section 2.3.
1.1.6. Contact Facility Manager Duncan Gascoyne (or in his absence Demin Li), or the NDCLS Facilities Manager Andrew Graham.

2.2 Normal working procedures

2.2.1. Access

2.2.1.1 Access to 4A10B is limited to authorised users who have been issued with a proximity fob. A list of authorised users is maintained and fobs are recalled when a staff member leaves. Staff will only be issued with a proximity fob on completion of mandatory training and competency sign-off by the Cryostorage Facility Manager.
2.2.1.2 Out-of-hours access to the adjacent corridor doors is by swipe card only. This is co-ordinated by the NDCLS Facilities Manager.
2.2.1.3 Within normal working hours (9am to 5pm) users **must** ensure that a colleague is aware that the Cryostorage Facility room is in use and is available to assist if necessary. There is no requirement to sign in and out of the room.
2.2.1.4 Outside normal working hours users must ensure that at least two members of staff are present in or immediately near (within calling distance of) the Cryostorage Facility room whilst work takes place. **Lone working in the Cryostorage Facility is not permitted under any circumstances.**
2.2.1.5 Exiting the room is by push-button (white box in diagram below) and is not card controlled.

2.2.2. Exterior checks

2.2.1.1. Prior to entering, check the control panel situated outside the door (blue LCD, below left) to make sure the oxygen level in the room measured by the two oxygen monitors (black boxes in photos above and below right) is not below 20%. **Do not enter the room if the oxygen level is below 20%, and report the problem to either the facility manager (Duncan Gascoyne) or the NDCLS Facilities Manager (Andrew Graham).**

2.2.1.2. Check that the blue warning light above the door (see below) is not flashing and/or that the alarm is not sounding.

2.2.1.3. If all checks are satisfactory enter the room using proximity tag.

2.2.2. Interior room checks

2.2.2.1. The oxygen alarm itself is mains powered while the sensors (QFM330) are battery powered. They last at least 2 years and should be replaced at the annual service. However, if the audible low battery alarm is activated, report it immediately to Duncan Gascoyne or Andrew Graham.

2.2.2.2. On entering the room, activate the extraction fan by pressing the green override button to the left of the exit door as you go into the room. The fan will remain active for 30 minutes and then switch off automatically if the oxygen level is above 20%.


2.2.2.3. Should the Oxygen level drop below 20%, the extraction fan will activate automatically, and speed of extraction will increase should oxygen level fall below 18%. The fan will continue to run for a minimum of 30 minutes.

2.2.3. Personal Protective Equipment

2.2.3.1. Personal protective equipment (PPE) is available for users of the Cryostorage Facility. This is stored within the Cryostorage Facility is and checked on a monthly basis by the Banham Group Laboratory Safety representative to ensure it is fit for purpose. Any defects in PPE should be reported immediately to either the Cryostorage Facility Manager or the NDCLS Facility Manager.

2.2.3.2. PPE includes: Protective blue apron (see below), Full-face visor (see below), Eye-protection (Safety glasses), Insulated gloves (leather insulated gloves with elasticated cuffs and blue insulated gauntlets such as those below). **NOTE:** blue insulated gloves are for use when handling frozen vapour-phase material only, NOT for handling liquid nitrogen


2.2.4. Removing or adding a sample from/to a storage tank

2.2.4.1. **NOTE:** There is a small but significant risk of a cryovial exploding once it is removed from LN_2 storage tanks, so all persons handling frozen tubes must wear full-face protection together with a laboratory coat. Users must not lift their visor to read a label on, for example, a cryovial.

2.2.4.2. Ensure that all required protective equipment is being worn and that the stainless steel bucket (see below) is present to hold the rack of frozen samples temporarily.

2.2.4.3. If transferring samples to/from the large tank, ensure that the Kik-step (see below) is present and in good condition (top rubber surface intact).

2.2.4.4. Ensure that you have ready a container with a lid in which to transport the newly-removed sample, in order to contain any explosion risk, and a pair of forceps to transfer the vial(s).

2.2.4.5. All vials must be clearly labelled with owner's name or initials, date of sample and sample identity.

2.2.4.6. Ensure that whenever possible the exact location for transfer is known by referring to accurate sample location records, which will minimise risk by reducing time taken to undertake transfer.

2.2.4.7. **NOTES** on manual handling samples from the large tank which may require working at height using the Kik-stool:

2.2.4.7.1. There are recommendations as to what men and women can be expected to lift relative to the position of the load, and the distance from the ground, and the distance from the body (see Figure below)

2.2.4.7.2. Minimise transport distance by bringing the stainless steel bucket as close as possible to the storage vessel

2.2.4.7.3. Make sure that clothing and footwear is suitable (particularly footwear with good grip)

2.2.4.7.4. Ensure that posture is as good as possible with feet slightly apart. Move feet whilst transporting the tower, do not twist your body. If you have any doubt that you can lift the tower (for example have special requirements such as musculo-skeletal problems or pregnancy), ASK FOR ASSISTANCE from another member of staff

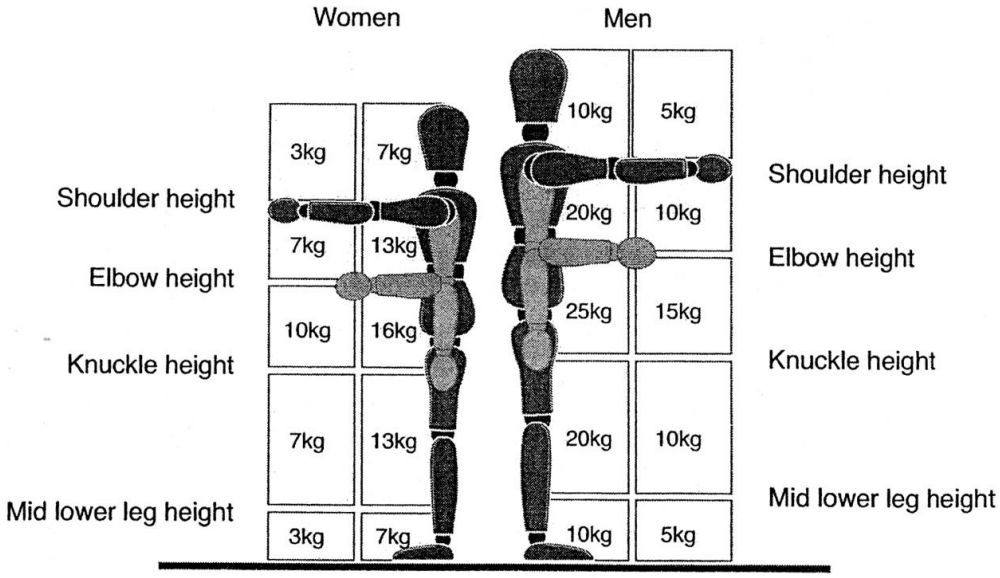


Figure 2 Lifting and lowering

2.2.4.8. Remove the storage rack from the storage vessel and place in the stainless bucket

2.2.4.9. Remove any retaining rods to a safe place

2.2.4.10. Remove the desired tray and transfer vial(s) as required to closed transport container. Replace each tray before removing another.

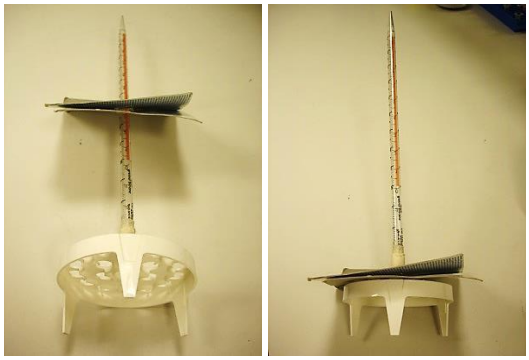
2.2.4.11. Replace the final tray into the tower and replace retaining rod. Return tower to storage vessel in the correct orientation ensuring correct fit and good closure of the vessel lid.

2.2.4.12. **NOTE:** if any samples are dropped into the liquid nitrogen users MUST obtain assistance from another user before attempting to collect the sample (samples must only be retrieved by using long tweezers in the smaller storage vessel - if samples are dropped in the large storage vessel they cannot be recovered)

2.2.5. Snap-freezing biological samples

2.2.5.1. Snap-freezing reduces the chance of water present in a biological sample forming ice crystals during the freezing process, and can be required to maintain sample integrity. In the case of tissue or lysates, snap freezing slows the actions of proteases and nucleases to inhibit degradation of molecules such as RNA or proteins.

2.2.5.2. Snap-freezing samples in medium storage containers: ensure that samples are held in a container (see pictures overleaf) which allows access of liquid nitrogen to sample, but does not itself hold liquid nitrogen to minimise risk to the user in the event of a tube exploding. Left picture below shows position to insert sample tubes into container, right picture shows position in which to immerse samples. Remove lid from storage vessel and immerse samples in liquid nitrogen until frozen. Transfer immediately to storage.

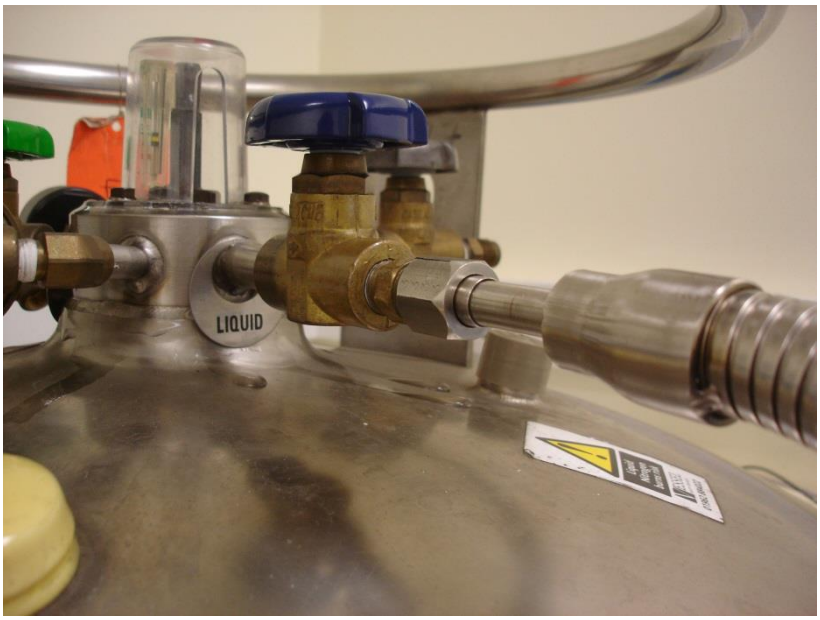


2.2.5.3. Snap-freezing samples in small storage flasks (Thermo-Flask type). Decant liquid nitrogen carefully from supply vessel to flask, immerse sample until frozen and immediately transfer to storage.

2.2.6. Filling/Topping-up a liquid nitrogen storage tank with liquid nitrogen

2.2.6.1. Wearing appropriate PPE, check that the hose is correctly attached to the liquid valve of the bulk storage cylinder tank (left picture below) and that, if present, the vessel brake is on (middle picture below). Place the nozzle into the cell bank to be filled, slowly open the liquid valve (blue valve in right picture below and overleaf), and add LN_2 to a depth of about 12cm (measured using a dip stick). This depth ensures that all samples are in the vapour phase. Close the liquid (blue) valve.

2.2.6.2. When the bulk storage cylinder tank is empty, close the liquid valve by hand and, whilst holding the brass nut on the fixed part of the vessel with the 19mm ring spanner provided, use the adjustable spanner provided in the facility to gently unscrew the hose clockwise to disconnect it from the tank.



2.2.7. Sending the storage vessel for refilling

When refilling of a bulk storage cylinder tank is required, the tank must be left on Level 0 for collection by NHS Estates staff. Wheel the tank into the lift and, using the lift over-ride key (which is kept in the Cryostorage Facility) to ensure an uninterrupted journey, send the tank down to Level 0. **PEOPLE MUST NEVER ACCOMPANY THE TANK IN THE LIFT** even when the tank appears to be empty. Go down the stairs to Level 0 to remove the tank from the lift. It is returned to level 4 by JRH Estates staff.

2.3. First Aid procedures

In the event of an incident requiring first aid, the procedures below must be followed. If medical treatment is required at A&E, take a copy of the 'Treatment of Cryogenic Burns' poster with the patient. All accidents/incidents/near misses must be reported. **Do not put yourself, or others around you, at risk in an attempt to administer First Aid treatment**. If in doubt, call for assistance.

2.3.1. Cryogenic Burns

Cold burns and frostbite should receive medical treatment as quickly as possible. The aim of the treatment is to raise the temperature of the affected part back SLOWLY back to normal.

2.3.1.1. Minor injuries treatment

2.3.1.1.1. Flush the area with TEPID water. Do not use a forceful flow of water as this could cause tissue damage.

2.3.1.1.2. Do not use hot water or apply any form of direct heat.

2.3.1.1.3. Move the casualty to a warm place (approx. 22°C) and seek medical attention

2.3.1.2. Major injuries treatment

2.3.1.2.1. Call for assistance **(from a hospital phone dial 4444)**

2.3.1.2.2. While waiting for medical attention/ambulance continue to flush the affected area of the skin with tepid water

2.3.1.2.3. Loosen the casualty's clothing and remove any tight jewellery

2.3.1.2.4. Keep the patient warm and at rest

2.3.1.2.5. Do not allow the patient to smoke and do not offer hot beverages or alcohol

2.3.2. Asphyxia

The release of nitrogen into the atmosphere reduces oxygen levels and causes hypoxia, which can be sudden or gradual.

- **Sudden asphyxia:** In sudden asphyxia (inhalation of gas containing little or no oxygen) unconsciousness is immediate and death follows quickly, unless action is taken.
- **Gradual asphyxia:** Gradual asphyxia can occur if nitrogen gradually displaces atmospheric oxygen. Unconsciousness and death may follow. The risk of gradual asphyxiation is particularly acute because the affected individual will not be aware of the development of dangerous levels of hypoxia.

2.3.2.1. First Aid for asphyxiation

2.3.2.1.1. If safe to enter, move the casualty to fresh air. If unconscious, open the airway, check breathing and pulse, call for help from a First-Aider **(or from a hospital phone dial 4444)** and be ready to resuscitate.

3. Training Requirements

In order to obtain a proximity fob allowing access to the NDCLS Cryostorage Facility, users must complete the NDCLS-run Safe Use of Cryogenic Liquids training course which covers both University policy and local rules. Users must also receive 'on-the-job' training in handling cryogenic liquids and be certified as competent to do so by Demin Li or Duncan Gascoyne before working independently. Any related SOPs and Risk Assessment documents must also have been read prior to undertaking work in the Cryostorage Facility. In addition, users are recommended to complete the Introduction to Manual Handling course run by the University Safety Office.
https://www.admin.ox.ac.uk/safety/safetytraining/trainsubj/manhand/

4. Staff Record of Acknowledgement

By signing below, I agree that:

4.1 I have read and I understand the contents of this document and will work in accordance with this SOP

4.2 My supervisor/manager agrees that, subject to having received any further relevant training for procedures associated with this SOP, I am able to perform work covered by this document

4.3 I understand that further 'on the job' or other training/supervision may be required before working independently

4.4 I understand that I may discuss my needs with my supervisor or line manager

Trainee				Supervisor/manager (if applicable)	
Name	**Position**	**Signature**	**Date**	**Signature**	**Date**

Trainee				Supervisor/manager (if applicable)	
Name	**Position**	**Signature**	**Date**	**Signature**	**Date**