Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

1. PURPOSE

This SOP describes how to safely transport, store and handle liquid nitrogen for use in liquid nitrogen cryostorage units.

2. <u>SCOPE</u>

This SOP applies to all users within the CBE laboratories that handle liquid nitrogen or maintain cells in cryostorage units.

The SOP describes the procedures for:

- Filling cryostores with liquid nitrogen (Section 5.1)
- Collection and storage of liquid nitrogen supply (Section 5.2)
- Emergency response (Section 5.3)

The SOP also includes notes on the Health & Safety aspects of liquid nitrogen (Section 5.4).

Read this SOP in conjunction with <u>SOP031</u> (Cryopreservation and Storage of Mammalian Cells), <u>SOP032</u> (Resuscitation of Cryopreserved Mammalian Cell Lines), <u>SOP036</u> (Maintenance of a Quality Laboratory Environment) and <u>SAF/CBE/007</u> (Use of Liquid Nitrogen in CBE Laboratories).

3. <u>RESPONSIBILITES</u>

Authorised CBE Laboratory Users

- Shall understand the health and physical hazards of cryogenic liquids (you must read <u>SAF/CBE/007</u> and <u>Section 5.4</u>).
- Shall undertake proper training with the Laboratory Manager or an authorised deputy.
- **Shall** ensure their work with liquid nitrogen does not create additional hazards for other CBE laboratory users.
- **Shall** inspect local oxygen monitors to ensure they are in good working order **before** handling liquid nitrogen.

Responsible Persons (RP)/Laboratory Manager (LM)

 Shall designate and organise training for CBE laboratory users who are required to use liquid nitrogen. Training must be recorded using the forms (<u>FSOP013.1</u> and <u>FSOP013.2</u>) which are

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

then placed into the individual's training folder as proof of authorisation. It is the RP/LM's responsibility to ensure that **only** authorised CBE users handle liquid nitrogen.

- **Shall** routinely maintain the liquid nitrogen fill level of **all** cryostorage units located in the CBE Laboratories as outlined in <u>Section 5.1</u>.
- Shall manage the supply of liquid nitrogen contained within 3 x 25 L Dewars located in Gas Pod 3 (<u>Section 5.2</u>)
- **Shall** ensure necessary controls (cryogenic personal protective equipment) are available and implemented to minimise the quantity and likelihood of liquid nitrogen spills and exposure to liquid nitrogen or its gaseous form.
- **Monitor** the handling of cryogenic liquids in accordance with good work practices (outlined in <u>Section 5.4</u>).
- **Provide** accessible operational instructions to authorised users of liquid nitrogen.
- **Maintain** a list (and contact details) of responsible person(s) and authorised liquid nitrogen users adjacent to the liquid nitrogen store in Gas Pod 3 and cryostorage units.

4. EQUIPMENT AND MATERIALS

- Cryolab 25 (25 L) Nitrogen Dewars (4 units) housed on Tipper Trolleys in Gas Pod 3.
- Biorack 750 cryostorage units (9 units) including Roller bases and padlocks/keys stored in H30/H31 & H34.
- Oxygen monitors.
- Metal spill trays (2 trays).
- Cryogenic PPE (Face visor or safety goggles, heavily-insulated gauntlet gloves, covered footwear, laboratory coat).
- 2 L Dewar flask
- Dipsticks
- Flasks

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

5. PROCEDURE

5.1 Filling cryostores with liquid nitrogen

This procedure describes the steps necessary to regularly supply with liquid nitrogen to maintain their cryogenic temperature.

NOTE: This procedure is a <u>2 person task</u>. Tasks are split between person 1 (<u>P1</u>) and person 2 (<u>P2</u>) as specified. Cryostores **should** be checked and filled **twice** weekly (usually Tuesday and Friday).

5.1.1 Setup

- a. (**P1/P2**) Collect the keys for the back door, Gas Pod 3 and the cryostorage units.
- b. (**P1/P2**) Prop open the back door and the autoclave room doors, then place "liquid nitrogen in use, do not enter" signs upon the autoclave room doors.
- c. (P1) Make sure oxygen monitors are in place and functional.
- d. (**P1**) Deploy the metal spill tray in the Autoclave room.
- e. (P1) Unlock the cryostore units.
- f. (<u>P2</u>) Retrieve a liquid nitrogen Dewar with Tipper trolley from Gas Pod 3 and place upon the spill tray in the Autoclave room (H31).

NOTE: It is the responsibility of (<u>P2</u>) to confirm there is sufficient liquid nitrogen supply within the 25 L Dewar stock to perform the filling procedure outlined in <u>Section 5.1</u>. If only one 25 L Dewar contains liquid nitrogen, it is recommended that the liquid nitrogen resupply procedure be followed (<u>Section 5.2</u>) **before** filling the cryostorage units.

g. (P1) Don the necessary PPE (See <u>Section 4</u>).

NOTE: Do not wear nitrile gloves underneath the heavily-insulated gauntlets). Ensure the Face visor or safety goggles are correctly positioned and secure before proceeding to the next step.

5.1.2 Check cryostorage fill level

For each cryostore unit:

- a. (P1) Retrieve the cryostore unit .
- b. (P1) Slowly remove the cryostore lid and safely store upright on the spill tray.
- c. (P1) Retrieve a dipstick and quickly place into the hole located in the base of the cryostore unit.
- d. (P1) Once the dipstick is correctly located, wait 5 seconds and then quickly remove the dipstick.
- e. (**P1**) Read the fill level out to **P2**.
- f. (P2) Record the date; fill level and operators on the cryostore unit-specific log sheet located upon the Autoclave room door.

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand
	5 5	11 5

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

- g. **lf:**
 - I. The fill level is \geq 19 cm **then** the cryostore does not need filling, so replace the cryostore lid and return the cryostore to its storage location and ensure antenna is upright on monitor.
 - II. The fill level is 15 19 cm **then** the cryostore needs filling with liquid nitrogen up to 19 cm (Section 5.1.3).
 - III. The fill level is < 15 cm **then** a deviation below the minimum fill level has occurred and the cryostorage unit should be filled immediately. A CAPA must be done by the RP/LM or a designated authorised user and submitted to the quality manager (QM) for assessment.

5.1.3 Filling cryostorage units

- a. (<u>P2</u>) Determine the amount of liquid nitrogen required to bring the level to 19 cm (1 cm fill volume = half a Dewar flask or 1 L).
- b. (P1) Place a funnel in the neck of the cryostorage unit to be filled.
- c. (<u>P1</u>) Slowly fill the 2 L Dewar flask with liquid nitrogen from a 25 L liquid nitrogen Dewar using the tipper trolley.

NOTE: Hold the flask securely at the neck. You **should** initially pour a small amount of liquid nitrogen and allow it to boil off to ensure the 2 L flask is cooled enough to minimise the risk of a liquid nitrogen spill due to rapid boil off.

- d. (<u>P1</u>) Carefully pour the liquid nitrogen into the cryostorage unit via the funnel so as to avoid splashing (smooth and steady pouring is best).
- e. (<u>P1</u>) Repeat steps <u>5.1.3 c d</u> as instructed by (<u>P2</u>) to dispense the required amount of liquid nitrogen to bring the fill level to 19 cm.
- f. (P1) After filling, remove the funnel and then check the fill level as outlined in Section 5.1.2 c e.
- g. (P2) Record the new fill level on the cryostore unit-specific log sheet.
- h. If
- a. The fill level is 19 cm then the cryostore is sufficiently filled so continue to <u>Section 5.1.3 i</u>.
 b. The fill level is ≤ 20 cm then repeat <u>Section 5.1.3 a h</u>.
- i. (P1) Allow the residual liquid nitrogen vapour on the funnel to boil off before removing the funnel.
- i. (**P1**) Replace the cryostore lid.
- k. (**P1**) Relocate the cryostore unit to its designated storage location.

REMINDER: All cryostorage units must be filled to the **maximum working volume (19 cm)**, relocated to their designated storage locations and the fill data logged before moving to <u>Section 5.1.4</u>.

5.1.4 Tidy up

- a. (P2) Relocate the 25 L liquid nitrogen Dewar from the Autoclave room to Gas Pod 3.
- b. (P1) Store the 2 L Dewar flask with the funnel in the Autoclave room.
- c. (P1) Relock all the cryostores using the cryostore keys.
- d. (P2) Lock Gas Pod 3 with the Gas Pod 3 key and then lock the back door.
- e. (P1/P2) Remove the "liquid nitrogen in use, do not enter" signs from the Autoclave room doors.

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

- f. (**P1**) Relock the outer Autoclave room door.
- g. (P2) Tidy the data sheets and replace in the plastic sleeve attached to the outer Autoclave room door.
- h. (**P1**) Remove, clean and store cryogenic PPE (Face visor or safety goggles, apron and heavilyinsulated gauntlet gloves) in the Autoclave room.
- i. (P1) Don nitrile gloves then store the rubber-lined metal spill tray in the Autoclave room.
- j. (**P2**) Replace the back door, Gas Pod 3 and cryostore keys.

5.2 Collection and storage of liquid nitrogen

5.2.1 Storage of liquid nitrogen delivered by BOC (UK).

This procedure describes the procedure for receiving liquid nitrogen delivered from BOC in a cryogenic tanker.

- a. Collect the back door and Gas Pod 3 keys.
- b. Unlock Gas Pod 3 and remove the 4 x 25 L liquid nitrogen Dewars from the Gas Pod.
- c. Monitor the filling of each Dewar by the BOC employee.
- d. Place each Dewar back in Gas Pod 3 after filling is complete.
- e. Lock Gas Pod 3.
- f. Return the keys to the back door and Gas Pod 3.

5.2.2 Collection of liquid nitrogen from the Physics Department (Backup supply).

This procedure describes the procedure for the collection and storage of liquid nitrogen from the backup supply located in the Physics Department.

Note: This is a **minimum 2 person** task, where 3 individuals are required if all 3 x 25 L liquid nitrogen Dewars are to be filled.

Only operators trained by the Physics Department or a named deputy may access the Physics Departmental liquid nitrogen supply.

- a. Collect the back door and Gas Pod 3 keys.
- b. Unlock Gas Pod 3 and remove the 25 L liquid nitrogen Dewars for filling from the Gas Pod.
- c. Walk to the Physics Department courtyard (Sir David Davies [W] building) off University Way) with the 25 L liquid nitrogen Dewars.
- d. One trained operator must go to the Physics Office to obtain (1) the key to the locked storage cage containing a 150 L liquid nitrogen storage tank and (2) a carbon paper pad to note the date, department, operator name and amount of liquid nitrogen taken for Physics own record keeping.
- e. Unlock the storage cage.
- f. The trained operator **must** don a Face visor or safety goggles and insulated gauntlets located in the storage cage.
- g. The trained operator then fills each 25 L liquid nitrogen Dewar from the 150 L dispensing tank.

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

Caution: The Dewar is filled from a metal hose on each pressurised tank. **Always** ensure the hose is located correctly inside the 25 L Dewar **before** opening the release valve on the tank.

Caution: Liquid nitrogen boil off will cause "blow back" of cold vapour from the neck of the 25 L Dewars at the start of filling.

Caution: The release valve may become frozen shut during prolonged filling. Regularly **manipulate the valve** during filling to avoid this.

Caution: After filling, the metal hose will be brittle. Do not excessively bend the hose during retrieval from a 25 L Dewar.

- h. Close the release valve and record the amount of liquid nitrogen dispensed in the log book next to the 150 L storage tank.
- i. Relock the storage cage.
- j. Note down the date, department, operator name and amount of liquid nitrogen on the carbon copy pad.
- k. Return the storage cage key and the carbon copy pad to the Physics Office.
- I. Carefully walk the filled 25 L liquid nitrogen Dewars back to Gas Pod 3 (Garendon Wing, Holywell Park).
- m. Lock Gas Pod 3 then return the keys.

5.3 Emergency response procedures

5.3.1 Liquid nitrogen spill response

If the volume of liquid nitrogen spilled is < 100 ml (**Minor Spill**) and correct procedures have been followed; then spill will be in a well-ventilated area and may be allowed to evaporate.

- a. Move any other personnel away from the spill area.
- b. Prop open doors to the laboratory if additional ventilation is required (determined by oxygen monitor alarms, see <u>Section 5.4.2</u>)
- c. If oxygen monitors are alarming (<18 % O₂) then immediately evacuate the area and contact the Laboratory Manager and Departmental Safety Officer.

If the volume of liquid nitrogen spilled is \geq 100 ml (**Major External Spill**) and external to the CBE laboratory then:

- d. Immediately evacuate the area.
- e. Cordon off the spill area and prevent any individual (whether staff member, student or general public) from accessing the spill area.
- f. Allow the liquid nitrogen to evaporate into the atmosphere.
- g. Contact the Laboratory Manager and Departmental Safety Officer.

If the volume of liquid nitrogen spilled is \geq 100 ml (**Major Internal Spill**) within the CBE laboratory then:

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

- h. All personnel must immediately evacuate the surrounding area or risk death from asphyxiation.
- i. Immediately contact the Laboratory Manager and Departmental Safety Officer.
- j. If the spill is very large (> 10 L) and/or in an enclosed space, a complete evacuation of the building may be necessary.
- k. The Fire Service should also be alerted to the situation if there is serious risk of combustion.
- 5.3.2 Oxygen depletion response

If the oxygen monitors are alarming and/or there is visible evidence of a liquid nitrogen spill then:

- a. Follow the appropriate spill response (<u>Section 5.3.1</u>).
- b. **Do not enter** or allow others to enter the spill location until (1) the atmospheric oxygen concentration has returned to normal (19.5 %); (2) there is no sign of spilled liquid nitrogen remaining; and (3) there is no risk of cryogenic burns from chilled surfaces in the local area.
- c. Contact the Laboratory Manager and Departmental Safety Officer immediately.
- 5.3.3 Fire alarm response

If the fire alarm sounds while you are working with liquid nitrogen then:

- a. Make safe the liquid nitrogen if possible before evacuating.
- b. If it is not possible to make safe the liquid nitrogen, then evacuate the building and immediately inform the Fire Brigade to the risks posed by liquid nitrogen (oxygen depletion and combustion in particular).
- 5.3.4 First aid response

Exposure to liquid nitrogen or cold vapour will cause Frost Bite to varying degrees. Treatment of such injuries all aim to restore tissue slowly back to normal body temperature (37°C). Short-term contact may be treated as minor as follows:

In the case of Minor Frost Bite:

- a. Move the injured person to a comfortable room if possible
- b. Remove or loosen clothing which may constrict blood flow to the frozen area.

Caution: Do not remove adherent clothing until the area has been thoroughly thawed

c. Place the affected area in tepid water or flow tepid water (40°C to 42°C) over the area for half an hour. Skin should change from pale yellow through blue to pink or red during this process.

Caution: Hot water (45°C) or dry heat should not be used. **Caution**: **Do not** rub the affected area during or after treatment.

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

- d. The injured person should not smoke or drink alcohol.
- e. Cover the affected area with bulky dry sterile dressing.
- f. Send the injured person to hospital.

Prolonged contact with liquid nitrogen may cause serious burns and blood clots requiring more sophisticated medical treatment. **Any** individual with a severe burn or who had liquid held in contact with the skin by gloves or clothing **should immediately** seek medical attention.

In the case of Major Frost Bite:

- h. Call for an ambulance.
- i. Follow the procedure for Minor Frost Bite (Section 5.3.4) as much as possible.

In the case of significant body exposure, the injured person must be re-warmed without delay.

g. The injured person should be placed in a bath of warm water at a temperature between 40°C and 42°C.

Caution: The temperature should be maintained within this range to ensure maximal rate of warming.

Note: If the facilities for this treatment are absent, then the injured person should be taken to a warm environment and lightly covered with one or two blankets until recovery is complete.

Caution: The injured person may go into shock during rewarming.

Note: Frozen tissues are often painless and appear waxy with a pale colour. Thawing after deep burns will result in vasodilation, increased capillary permeability and oedema. Tissues will become painful, swollen and vulnerable to infection after thawing. Thawing may take 15 – 60 minutes and should be continued until the pale colour of skin turns to pink or red. The thawing process may require major analgesia. Symptomatic treatment and the prevention of infection are indicated.

Caution: If the frozen body part is thawed by the time medical attention is obtained, do not re-warm. Cover the area with dry sterile dressing and with a large bulky protective covering.

In the case of Suffocation:

If a person becomes dizzy or loses consciousness while working with liquid nitrogen, follow the procedure below:

- h. **Immediately** move the person to a well-ventilated area.
- i. Determine whether the person is still breathing.
 - I. If breathing has stopped, apply artificial respiration and call a first aider
 - II. If the person is still breathing, call a first aider.
- j. Call for emergency medical assistance.

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

5.3.5 Incident Reporting procedure

All incidents involving spillage of liquid nitrogen **must** be reported to your immediate supervisor or laboratory manager.

It is University Policy that a full Incident Report **must** be written and submitted to the Area Safety Advisor immediately after the spillage is resolved.

5.4 Health & safety aspects of liquid nitrogen use

5.4.1 Hazards and risks

- a. <u>Asphyxiation</u>: When liquid nitrogen boils off, 1 cubic litre is converted to 683 litres of gaseous nitrogen. If this occurs in an enclosed space, oxygen displacement will occur which can cause suffocation without warning.
- b. <u>Explosion</u>: Liquid nitrogen boil off inside a sealed container is an explosion risk. Commonly used cryovials are not hermetically sealed, and liquid nitrogen may enter vials during storage which subsequently explode upon rapid warming.
- c. <u>**Combustion**</u>: Displacement of oxygen during liquid nitrogen boil off can concentrate oxygen, which is highly flammable. Condensation promoted by liquid nitrogen can also promote oxygen enrichment of liquids which increase their risk of combustion.
- d. <u>Cold</u>: Liquid nitrogen is extremely cold (-197°C), which presents immediate risk of cold burns, frostbite and hypothermia from liquid and the resultant vapour. Transient exposure to cold gasses can make breathing difficult and provoke asthmatic episodes in susceptible individuals.
- e. <u>Embrittlement</u>: Liquid nitrogen can chill many materials (e.g. carbon steel, plastics and rubber) such that they become brittle and prone to fracture under previously tolerable stress loads.

5.4.2 Control strategy

- a. <u>Oxygen Monitoring</u>: Low oxygen sensors are located in the CBE Laboratories for use with liquid nitrogen. They continuously monitor local oxygen concentration and will alarm if the oxygen concentration drops as a consequence of oxygen displacement via liquid nitrogen boil off.
 - Atmospheric oxygen should be \geq 19.5 %
 - If oxygen is ≥ 18 % and < 19.5 % then increase ventilation or decrease the amount of liquid nitrogen in the local environment.
 - If oxygen is < 18 % evacuate the local area **immediately** and contact the LM/RP and Departmental Safety Officer (DSO). No person should enter the area without air supplied breathing apparatus.

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

- b. <u>Acceptable containment</u>: Use only approved unsealed containers (i.e. specially designed for cryogenic fluids).
 - Do not use domestic vacuum containers (e.g. Thermos flasks)
 - Do not use sealed containers
 - Do not store liquid nitrogen in fully uncovered containers for prolonged durations (> 1 minute)
 - Keep liquid nitrogen containers clean and free of oil, grease or other materials which may become hazardous in contact with cryogenic fluids or condensed oxygen.
 - Do not transport liquid nitrogen in enclosed vehicles (use dry-shippers instead).
 - A wheeled Dewar/Tipper Trolley must be used to transport liquid nitrogen between buildings on foot.
 - If moving liquid nitrogen between floors, a Goods lift should be used. Personnel must not travel in a lift containing liquid nitrogen Dewars.
 - If a Goods lift is unavailable, you must ensure no persons accompany vented Dewars within a passenger lift.
- c. <u>Personal protective equipment</u>: Suitable protective equipment <u>must</u> be worn <u>at all times</u> when handling liquid nitrogen or touching object and surfaces cooled with liquid nitrogen.
 - A face shield or safety goggles to protect the eyes
 - A pair of **heavily-insulated gauntlet gloves** to provide <u>short-term</u> protection from accidental contact with liquid nitrogen. Do **not** wear standard laboratory gloves beneath these gauntlet gloves. Ensure the gauntlet gloves are dry and intact (no holes). Do **not** assume gauntlet gloves will protect you from prolonged contact with liquid nitrogen.
 - Wear covered protective footwear (i.e. no open topped shoes/sandals). Ensure liquid nitrogen is not splashed or spilled onto the floor. No footwear will resist prolonged contact with liquid nitrogen.
 - Wear a **laboratory coat**. Ensure the coat is <u>fully buttoned up</u>. Ensure liquid nitrogen is not splashed or trapped in clothing near the skin. Trapping of liquid nitrogen in this way increases the severity of injuries caused by liquid nitrogen.
- d. <u>Handling guidelines</u>: When directly handling (e.g. pouring) liquid nitrogen using the procedures outlined in this SOP, the following guidelines apply:
 - Minimise boiling and splashing of liquid nitrogen when dispensing. <u>Do not rush</u>, and minimise personal risk by standing at a <u>reasonable distance</u> from the site of boil off.
 - **Do not allow** unprotected body parts to have contact with non-insulated pipes or vessels containing liquid nitrogen. Flesh may freeze to externally cold metal surfaces and tear when attempts to withdraw are made.
 - Use tongs, long insulated forceps or long-handled sieves when handling objects which are being immersed in liquid nitrogen.
 - If immersing sample tubes and vials into liquid nitrogen, **minimise the explosive risk** by <u>gently venting</u> vessels shortly after removal from liquid nitrogen.

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

- **Be gentle** with extremely cold objects, as the <u>risk of fracture</u> is increased at low temperatures.
- Never handle liquid nitrogen alone. It is mandatory that liquid nitrogen handling be carried with at least one other individual in attendance. A second individual can provide assistance if required and take appropriate action in case of emergency (see <u>Section</u> <u>5.3</u>).
- Never leave open containers of liquid nitrogen unattended.
- **Never** leave materials recently extracted from liquid nitrogen **unattended**.
- Always handle liquid nitrogen in a well-ventilated area.
- **Only** handle liquid nitrogen with containers specifically designed for the task.
- **Never** dispose of liquid nitrogen by pouring it on the floor.
- **Beware** of rapid boil-off when filling containers with liquid nitrogen.
- **Always** allow sufficient time for vessels receiving liquid nitrogen to cool down to ultra-low temperatures when pouring liquid nitrogen.
- Never seal liquid nitrogen in any container.
- **Never** dip hollow tubes into liquid nitrogen (as liquid might be forcibly ejected from the tube).

6. DOCUMENTATION

The following records are outputs of this SOP:

- FSOP013.1 Use & Handling of Liquid Nitrogen in CBE Laboratories Training Agreement
- FSOP013.2 Transfer of Liquid Nitrogen from Pressure Vessel Training Agreement
- FSOP013.3 Warning Do Not Enter Liquid Nitrogen In Use Notice
- FSOP013.4 Cryostorage Liquid Nitrogen Fill Log

Completed records are stored as following:

FSOP013.1 Stored in users training record FSOP013.2 Stored in users training record FSOP013.4 Stored in Lab Management Folder

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
001	14Oct2009 Reviewed by K.Brosnan	 Section 3 – added additional references to university policies Section 6.2 changed wording of PPE requirements from preferred to must be worn. Also added requirement to wear full length apron to step 6.2.3. Section 6.2.4. Altered use of 2 Litre flask to only be used for the transfer of LN2 from a storage Dewar to the cryostorage unit. Section 7.1 (iii) added signs warning LN2 in use must be up and visible to other personnel. (v) remove "/or" as these are a requirement not optional. (vi) Rephrased statement to show cryo- gloves are a requirement not optional. (vii) Added "do not use" to statement. Section 7.2.1 added note highlighting two person task. Added caution note regarding brittle metal hose. Section 7.2.2 added note highlighting two person task. (vi) Removed gloves from statement as these should not be worn at this time. Steps (vii – xv) rephrased procedure to make it more stepwise and clearer for the operator to understand. Section 7.2.3 added note not to reconnect cryogaurd for 30mins Section 7.3 added note to highlight two person task, included (i) to refer to SOP031 for full instruction before placing/removing a vial 	002
002	1 st Feb 2011 Reviewed by K.Brosnan	Annual Review – No amendments required	003
003	23/02/2011 P.Hourd	Revised sections to include the CBE Tissue Engineering Laboratory (T208B), located in the Wolfson School. Including Section 2, added 5.1(iii), added procedure for T208B (Section 7.3)	004
004	25/04/2014 A.Picken	Changed to Lean template. Complete rewrite of SOP in response to Change Note SRN012.	005
005	26/11/15	Review- No amendments required	006

Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand

Standard Operating Procedure

Title: SAFE USE AND MAINTENANCE OF LIQUID NITROGEN STORES

Location: CBE Laboratories

	J.Bowdrey		
006	A.Picken	Review - Ammended Liquid nitrogen fill height from 21 cm to 19 cm.	007
007	J.Bowdrey	Review- Changed number of Cryostores and Dewars	008
008	C.Kavanagh 8 th February 2022	Removed the section (carefully disconnect the temperature monitoring cable). Added ensure antenna is upright on monitor. Removed Brian Dennis as contact Added cleaning of face shield	009

Version 009	Effective Date: 08/02/2022	Review 08/02/2024
Written by: A. Picken	Reviewed by: J.Bowdrey	Approved by: P. Hourd/K. Sikand