

**OMNI Electron Microscopy
University of Otago**

**LIQUID NITROGEN SAFETY INSTRUCTIONS &
EMERGENCY RESPONSE PLAN**

To be read by all users of liquid nitrogen.

Emergency contacts

My departmental health & safety officer:

Name: _____

Telephone number: _____

Emergency Services Phone 1 - 111
(Fire Brigade and Ambulance)

University Security and University extension: 5000
Emergency number

Liquid Nitrogen Generator room and OMNI EM contacts

Liquid Nitrogen dispensing assistance:

Fatima Jorge Email: **fatima.esperancajorge@otago.ac.nz**

Phone: University extension: 7301

Niki Hazelton Email: **niki.hazelton@otago.ac.nz**

Phone: University extension: 7301

Liquid Nitrogen Generator room equipment; supervision & faults:

Richard Easingwood OMNI EM Technical Manager

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or call mobile: 021 279 7301

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A. Introduction

1. Permission to obtain and use liquid nitrogen (LN2).

Those intending to use LN2 must undergo a training session and be familiar with the associated hazards. Training will include the safe handling of LN2 and the appropriate emergency procedures involved.

It is recommended that users of liquid nitrogen undergo refresher training annually.

2. The hazards involved with using liquid nitrogen (LN2).

There are four major hazards associated with the use and handling of LN2.

1. **Asphyxia:** Nitrogen gas displaces oxygen in the air causing potentially lethal hypoxia. Each litre of liquid nitrogen expands to 0.7 cubic metre of gas. Therefore, as little as 7 litres, if vaporised, can completely displace the air in a passenger lift, and a smaller vaporised volume can result in a lethal drop in oxygen level. Liquid nitrogen must not be introduced to small, unventilated rooms or spaces (including freezers). In small rooms, the door should be left open while liquid nitrogen is being used. Liquid nitrogen must not be 'stored' in a freezer. This does not significantly reduce the boil-off rate from the dewar and can create a lethal, oxygen-free chamber (particularly in the case of chest freezers) that can lead to asphyxiation.
2. **Expansion explosion:** Because of the high expansion ratio between liquid nitrogen and nitrogen gas (~700x), liquid nitrogen must never be put in sealed containers. The pressure in a sealed container can quickly build to the point where the vessel explodes. **Without exception, all containers must have a loose fitting top or a vent hole, or a pressure release valve.** It is critical that the gas is allowed to escape as it boils off. This applies to all storage and transfer dewars, and any specimen containers such as cryovials. Even a small tube, such as a cryovial, will explode with dangerous force if LN2 evaporates inside it. NB: Normal cryovials can be made safe for submerging in liquid nitrogen by making a small hole in the lid, otherwise they must not be submerged in, or filled with, liquid nitrogen. Non-vented cryovials must only be stored in the gas phase inside a storage dewar.
3. **Freezing of tissue:** Contact with skin or eyes can result in serious injury. LN2 is extremely cold (-196°C). LN2 and the cold gas stream that comes from the liquid will rapidly produce frost-burn. Eyes are particularly vulnerable and permanent eye damage can result from contact with LN2.
4. **Asthma:** The inhalation of chilled air may trigger an asthma attack in susceptible individuals. Good ventilation helps reduce this risk.

3. Personal safety equipment.

Suitable protective clothing must be worn at all times when handling LN2 and cold nitrogen gas. Protective clothing includes:

1. **Eye protection.** A full-face visor is best however safety glasses with side protection are acceptable. Safety glasses without side protection are not acceptable.
2. **Insulated, gauntlet-type gloves.** Handling cold dewar pipes and valves should be done only when wearing insulating gloves. Gloves must be loose fitting so that they can be removed rapidly if liquid nitrogen accidental spills inside them.
3. **Suitable footwear.** Wear shoes that cover and protect the feet. Open shoes i.e. sandals and jandals, are not acceptable.
4. **A laboratory coat.**

4. Working with liquid nitrogen.

Before working with LN2 always have a safety plan in case of accidents. If the dewar you are using is knocked over, what will you do? Can you quickly and safely exit the room? What about others in the vicinity - can you warn them to evacuate? If someone passes out from oxygen depletion, or has an asthma attack, what will you do? Can you call for help quickly? Is there anyone nearby who will notice if you have problems?

And finally, how will you safely dispose of any liquid nitrogen that you have left over? Liquid nitrogen must **never** be poured down a sink as it will destroy the plumbing, causing leaks and a potentially explosive nitrogen blow-back. For small quantities, it can usually be left somewhere with good ventilation, with the dewar (loosely) covered, to evaporate. Always dry the dewar well before refilling it with LN2.

There is a danger of rapid oxygen depletion leading to asphyxiation when working with LN2. It is strongly advised that you never work alone with it, even when working with small quantities. A second person should always be in the immediate work area and this person must also be aware of the dangers. The handling, use and storage of LN2 must only be carried out in a well-ventilated area.

Where possible, the bench tops where LN2 is used should be slightly tilted so that any spilt LN2 flows away from, rather than towards, the user.

While dewars must not be tightly sealed, a loose-fitting top should be placed over dewars whenever possible. This reduces the formation of ice, and also reduces the danger from the fractional distillation of oxygen from the air. Oxygen condenses (liquefies) at a slightly warmer temperature than liquid nitrogen. As a result, where there is a lot of air flow across the top of an open dewar (as in a fume cupboard), a layer of liquid oxygen can slowly form over the nitrogen, with a resulting fire risk.

Care must be taken during the transfer of LN2 between vessels. Always allow the receiving vessel to cool down slowly to LN2 temperature. Boiling often occurs when LN2 is introduced into a wide mouth flask at room temperature. This boiling may result in LN2 splashing out from

the warm container and causing frost burns. When introducing LN2 into any narrow necked container at room temperature, 'blow-back' from the dewar neck may occur. This results from the rapid vapourisation and expansion of N2 gas inside the dewar. Add liquid nitrogen to warm dewars slowly!

5. Transport of liquid nitrogen.

The dewar used to transport liquid nitrogen, whether 1 litre or 30 litres, must appropriate for the task. Dewars should be regularly inspected for damage. Any external frosting or cold patches on the outside of the dewar (other than during filling) is a sign that the dewar's vacuum insulation has failed. This will result in a rapid loss of LN2 and potentially greater risk of asphyxiation.

The transportation of LN2 through publicly accessible areas requires special care, especially where there is an uneven surface or vehicle traffic to avoid.

Dewars containing LN2 should not be transported in a lift unless it is possible to prevent anyone from getting into the lift. There is a risk of asphyxiation if the lift becomes stuck. If transport in a lift is required it is recommended that at least two people are involved. One person loads the lift and sets it off, the other waits at the end point to receive the LN2 dewar. If the lift is travelling between several floors and cannot be locked, then there is a risk some one may get on the lift at the intermediate floors. In this case someone should monitor each floor in between to prevent anyone from entering the lift.

The manual lifting of large liquid nitrogen containers (more than 20 litres in size) requires two people. This is to prevent back injuries but also to provide help in the case of an accident. Each person must be wear suitable safety equipment including insulated gloves and full-face visor.

Large storage dewars should be periodically purged to prevent oxygen enrichment in the dewar. It is also a good idea to empty and dry out large storage dewars periodically to prevent ice build up inside.

B. Protocols in the Event of Spillage

a. Minor spill (500mL or less)

1. Liquid nitrogen must only be used in a well-ventilated area so any minor spills may normally be allowed to simply evaporate. Ensure good ventilation while evaporation is occurring (open doors and windows if possible).
2. Move all personnel away from the direct area of spillage to prevent any chance contact with liquid nitrogen.
3. Ensure no one enters the area until all liquid has evaporated and sufficient time has elapsed for gaseous nitrogen to be removed.
4. Inform your health and safety officer of the incident.

b. Major spill (more than 500mL)

Major spills are likely to result from either from a spillage from a hand carried transport dewar or from a larger dewar tipping over. Treatment of a major spillage is dependent on the location of the spillage whether inside or outside the building.

Interior spillage

Any large internal LN2 spillage must be treated very seriously and action taken immediately. Because of the rapid conversion of even a small amount of LN2 into a large volume of gaseous nitrogen (a 700-fold expansion) any enclosed area will be affected very rapidly by a depletion of oxygen, leading to possible asphyxiation and death.

1. All personnel must be **immediately evacuated** from the surrounding area.
2. Ensure that the area is secured off so no one can enter the spill area from any direction. The security cordon must be sufficiently distant from the spill area to ensure safety of the personnel on duty.

Consideration must be made for rooms above and below the spill area as well.

3. Contact your departmental spill team. Upon arrival the spill team will manage the response. See attached Appendix, Chemical Spills.

External spillage

1. **Immediately evacuate the spillage area and cordon it off at a safe distance.**
2. Ensure no one enters the spill area. The security cordon must be sufficiently distant from the spill area to ensure safety of the personnel on duty. Be aware of wind direction. Do not stand downwind of the spill.
3. Contact your departmental spill team.

C. First Aid

a. Skin contact – the treatment of cold burns and frost bite

- Remove any clothing that may be holding cryogenic liquid. Loosen any clothing that may restrict blood circulation.
- Do not apply direct or dry heat to the affected parts. If possible, place the affected part in tepid water (do not exceed 40°C), or flush with copious amounts of tepid water.
- Do not rub the affected area in an attempt to improve circulation as tissue damage may result.
- Keep the victim warm as shock may set in. Do not leave the victim alone.
- Send for immediate hospital attention for all but the most superficial injuries.
- Protect the frozen parts from further injury or from infection. Dry, sterile dressings can be used for the purpose but they should not be allowed to restrict blood circulation.
- Do not offer or allow alcohol or cigarettes.

b. Eye contact

- Flush with warm water (do not exceed 40°C) for about 15 minutes *and seek hospital attention*. Keep the victim warm.
- Do not offer or allow alcohol or cigarettes.

c. Treatment of asphyxiation

As a general rule, do not attempt to rescue anyone from a confined space if they were working with liquid nitrogen and have lost consciousness - raise the alarm, open the door and maximise ventilation to the room. Call the fire brigade (1-111).

A victim of asphyxiation may not be aware that they are being asphyxiated. If any of the following symptoms appear to affect a workmate in situations where liquid nitrogen is being used, the person needs to be moved to open air as quickly as possible:

- Rapid breathing
 - Nausea
 - Vomiting
 - Inability to move
 - Convulsive movements
 - Collapse
 - Fatigue
 - Faulty judgment
 - Insensitivity to pain
 - Abnormal emotions
 - Abnormal pulse
- Before taking action quickly assess the level of danger in the area to yourself.
 - **If there is no risk** of asphyxiation or burns to you then immediately move the affected person to the open air.
 - **If there is a risk** of asphyxiation or burns to you then call emergency services immediately (1-111). The Fire Brigade has the breathing equipment required to attempt a rescue.
 - If you are able to remove the victim from the area, keep them warm. Shock may set in. Obtain medical help as soon as possible.
 - If the victim has become unconscious, check for a pulse and breathing. If you cannot detect these, administer artificial respiration and ask someone to call an ambulance (1-111). Ask that person to inform you as soon as they have called the ambulance.
 - Ask that person to call your departmental spill response team.
 - If it can be done safely, shut off the source of the liquid nitrogen and ensure adequate ventilation to dissipate the gas.