

Code of Practice

SAFE USE OF LIQUID NITROGEN

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Royal Free Hampstead NHS Trust
&
Royal Free & University College Medical School
(Royal Free Campus)

Liquid Nitrogen - Code of Practice

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Liquid Nitrogen - Code of Practice

INTRODUCTION

This document covers the transport, storage, use and disposal of liquid nitrogen. It sets the standards expected within the trust.

HAZARDS

There are two broad types of hazard arising from liquid nitrogen (hereafter referred to as LN) – those related to temperature and those related to vapour.

TEMPERATURE-RELATED HAZARDS

LN has a temperature of -196°C .

Severe cold burns can result from skin contact with the liquid or objects cooled by the liquid or vapour.

The skin will freeze to cold metal surfaces.

Many materials become brittle at this temperature and can shatter or crack.

Liquid oxygen may condense in containers of LN. **This raises the possibility of an explosive reaction with oxidisable material.**

VAPOUR-RELATED HAZARDS

LN expands around 700-fold when it vaporises at room temperature (eg 1 litre of liquid produces nearly 700 litres of gas).

Closed vessels containing LN may explode because of the build-up in pressure caused by the evaporation.

In poorly-ventilated rooms there is a danger that air will be displaced by the nitrogen, leading to an oxygen-deficient atmosphere and death by asphyxiation.

Asphyxia – Effect of O ₂ Concentration	
O ₂ (vol %)	Effects and Symptoms
18-21	- No discernible symptoms can be detected by the individual.
11-18	- Reduction of physical and intellectual performance without the sufferer being aware.
8-11	- Possibility of fainting within a few minutes without prior warning. - Risk of death below 11 vol%.
6-8	- Fainting occurs after a short time. Resuscitation possible if carried out immediately.
0-6	- Fainting almost immediate. - Brain damage may occur, even if rescued.

The danger from very low oxygen levels (ca. 6% or less) is that you become unconscious after two breaths – you do not even have time to realise that something is wrong. If you remain in that atmosphere for a few minutes you die.

Multiple fatalities are common in such circumstances because a passer-by or colleague, seeing someone collapsed, rushes into the room to render assistance but succeeds only in becoming a casualty.

This type of event is not confined to a whole room. If the work area has a low-lying tank or other type of container in which gas can collect, then this, too, could be a source of danger. Anyone putting their face into the tank and breathing insufficient oxygen could become unconscious and subsequently die. (A few years ago an engineer inspecting petrol tanks in a garage forecourt died although he was in the open air. He put his head into a shallow inspection pit above an underground tank. The air in the pit was rich in nitrogen and poor in oxygen; he collapsed, falling forward into the pit; he was not missed until it was too late.)

PRECAUTIONS

In addition to emergency actions, for example following splashes or spillages, which must be prepared for, there are five main activities that could lead to danger:

- Transport
- Storage
- Filling
- Use
- Disposal

They are discussed in detail below.

RISK ASSESSMENT

The precautions that need to be taken will depend to some extent on individual circumstances and should be decided following a risk assessment (as required by both the Management of Health and Safety at Work Regulations 1999 and the Control of Substances Hazardous to Health Regulations 2002 (COSHH). As discussed below, some of the precautions are common to all users.

Appendix C shows a risk assessment proforma that deals with the hazards and precautions for the use of LN; it does not cover other aspects of the work and individual risk assessments should be tailored to suit the particular circumstances. The form in Appendix C should be regarded as an addendum to the normal risk assessment proformas.

Appendix D contains the trust standard for risk assessment.

TRANSPORT

INSIDE THE BUILDING

The transport of LN via the stairs is potentially dangerous. A spillage of LN, for example from the container being dropped or overturned, could result in cryogenic liquid flowing down many flights of stairs. See Appendix D for the trust standard.

If LN is transported in a lift with solid doors, the lift becomes a confined space as defined by the Confined Spaces Regulations 1997. The danger is that the lift will breakdown between floors and that the LN will boil off, displacing the oxygen in the lift, and causing the death of the people in the lift.

Hence it is not acceptable for people to travel in the lift with the LN dewar. Two lifts – numbers 7 and 52 - have been modified to allow dangerous goods to be transported without being accompanied. The procedure is described in Appendix B.

Appendix D contains the trust standard for use of lifts.

ROAD TRANSPORTATION OF SAMPLES IN LN

This is a potentially hazardous activity because of evaporation of nitrogen into the vehicle. The LN container should not be in the same compartment as the driver and passengers, which means

that hatchbacks, minibuses and most vans are not suitable. To avoid the effects of leakage of nitrogen gas from the storage compartment into the passenger compartment, the driver should keep a steady flow of air into the car. The standards in Appendix D apply.

STORAGE

The danger is one of asphyxiation from nitrogen gas displacing the air.

Outside the building, LN dewars are stored inside an enclosure with mesh walls. Hence there is ample natural ventilation.

Storage inside the building is more of concern. The greatest danger is in the morning, because there could have been significant build-up of asphyxiant overnight and especially over the weekend. Although the oxygen deficiency is unlikely to be hazardous under normal conditions (see table 1, Appendix A), there is still uncertainty about abnormal conditions, for instance the degradation of insulation leading to a larger-than-expected release of gas.

The following are all **undesirable features** for a room in which LN is stored:

- poor ventilation
- small size
- large volume of LN
- windowless door
- no oxygen deficiency alarm

See Appendix D for the trust standard.

FILLING

VAPOUR-RELATED

Approximately 10% of the top-up liquid evaporates during the process. So, a 25litre top-up produces 1707 litres of gas. From tables 2a and 2b in Appendix A it can be seen that filling has a much greater effect on oxygen levels than does normal storage. Table 2a shows the effect on a room with 0.4 air changes an hour – this is a typical figure for a room without mechanical ventilation. Table 2b shows the beneficial effect of increasing the air flow rate to 1 air change an hour.

TEMPERATURE-RELATED

Personal Protective Equipment (PPE) should be the last resort, but will almost always be needed when LN is used. See below for a description of PPE.

USE

In addition to concerns related to both temperature and vapour, there is a further **concern of infection**.

INFECTION

It has been reported in the scientific literature that LN tanks can become contaminated with viruses, bacteria, fungi and cells. In one incident, this contamination (Hepatitis B virus) was passed on to recipients of a bone marrow transplant. Although the samples in these reports were not stored in cryovials, the possibility must exist; if there is leakage of LN into a vial then it is reasonable to anticipate some transfer of material out of the vial into the surrounding liquid.

Hence the storage of cell lines from primary sources (which may be infected) in the same tank as cell lines from commercial sources should be avoided.

Storage of cryovials in the LN vapour phase is advised for good laboratory practice. Furthermore, the manufacturers of cryovials state that they should not be immersed in LN. This method also avoids contamination between samples.

VAPOUR-RELATED

oxygen depletion

The main concern is from a spillage. The undesirable characteristics of a room are as for storage. Table 3 in Appendix A suggests that LN should not be used in rooms where the ratio of the room volume (m³) to the LN volume (litre) is less than 15 unless there is a good ventilation.

explosion

Vessels containing LN should not be sealed because of the danger of explosion. Any caps/lids must be vented, with a sufficient aperture to prevent blocking by ice. Glass dewars should be taped so that in the event of an implosion, the glass fragments will be contained.

There have been incidents of exploding cryopreservation vials. If the vials are stored immersed in the liquid, it is possible for liquid to leak into the vial. When the vial is returned to room temperature, the LN inside it evaporates raising the pressure until the vial explodes. On the Royal Free campus explosions have been experienced with one particular design of vial. It features a female cap, with a fine thread and no sealing ring. This type of vial should not be used. No incidents have been reported with another design in which the cap is male, has a thicker thread and has a sealing ring.

TEMPERATURE-RELATED

Personal Protective Equipment should be the last resort, but will almost always be needed when LN is used. The parts of the body needing protection from splashes of LN will depend on how the LN is used.

DISPOSAL

Surplus LN should be allowed to evaporate naturally inside a fumecupboard or a well-ventilated area.

LN must not be poured down the sink or drain.

TRAINING

Training is needed for everyone handling / using LN.

PERSONAL PROTECTIVE EQUIPMENT

It should be remembered that PPE is not designed to withstand immersion in, or prolonged contact with, cryogenic liquids.

Face

Splashing should be regarded as likely during any pouring operation. The PPE options are:

1. goggles
2. full-face visor
3. full-face visor with a chin guard.

Note:

- Eye protection should be to BS EN 166 grade 3
- Safety spectacles do not give adequate protection against splashes are NOT suitable
- Goggles protect only the eyes. A visor protects the eyes and the face. However it is still possible for liquid to splash up underneath the visor; the use of a visor with a chin guard should be considered if this is likely.

Hands should be protected by special gloves (to BS EN 511 (cold protection)).

The possibility of LN being **splashed into the glove via the cuff area** needs to be considered in the risk assessment. Loose, standard-length, gloves are more susceptible to splashing, especially if one hand is holding the receiving vessel while the other tips the storage dewar. (Ideally this eventuality should be designed out of the process – eg by providing a firm holder for the receiving vessel.) Alternatives are long-length gloves or standard gloves with elasticated cuffs.

Body

If splashing on to the body is likely, an apron should be worn. It should be from non-woven fabric and not have pockets. It should be long enough to protect the lower part of the legs.

Feet also need protection. Open-toed shoes and sandals are not suitable. If boots are worn, trousers should be worn outside the boots, to prevent liquid running into the top of the boot.

See Appendix D for the trust standard on PPE.

APPENDIX A

OXYGEN DEPLETION ARISING FROM EVAPORATION OF LIQUID NITROGEN

Liquid nitrogen evaporates slowly and could, in certain circumstances, displace enough of the air from the room to make the atmosphere dangerous to people.

There are three main sources of gaseous nitrogen:

- natural evaporation from the storage dewar
- evaporation during dewar filling and top-up
- spillage of LN

NATURAL EVAPORATION

Of particular concern are mornings because several hours will have elapsed since the room was entered, doors opened, etc.

The following calculation and Table 1 give an approximate idea of the degree of danger. The important parameters are:

- Volume of the room
- Volume of LN in store
- The number of air changes per hour
- The rate of LN evaporation, which depends on the integrity of the insulation

The standard equation for calculating nitrogen gas concentrations is:

$$C_N = L / V_R * n$$

Where C_N = increase in gas concentration after a long period

L = gas release (m^3/hr); V_R = room volume (m^3); n = air changes/hour

1 litre of LN produces 683 litres of gas.

The evaporation rate depends on the particular dewar. Manufacturers quote figures of around 0.8% per day for 25 litre vessels and 1.5% per day for 10 litre vessels. It is widely accepted that these figures should be doubled to allow for insulation degradation.

Example: at 0.8%/day, a 25l dewar releases 11.4 litres of gas per hour. Table 1 shows the effect of normal evaporation on oxygen levels.

TOPPING-UP / FILLING

Top-up / filling loses around 10% of the topping up volume so: 25l top-up loses 2.5l LN, producing 1707 litres of gas.

So filling dewars has much more effect on oxygen depletion than does normal storage. Table 2a shows the likely effects for a range of LN volumes and room sizes. The calculations assume an air change rate of 0.4 per hour – this is a typical figure for a room without mechanical ventilation. Table 2b shows the effect of increasing the ventilation to 1 air change an hour.

SPILLAGE

Table 3 shows that spillage can have a dramatic effect on oxygen levels. For these calculations the effect of ventilation was ignored. Further assumptions are that the LN vaporises immediately and the released nitrogen gas mixes with the air. The figures therefore represent a pessimistic case.

Room Volume m3	Volume of liquid nitrogen, litres										
	10	25	50	75	100	150	200	250	300	400	500
15	21.0	21.0	20.9	20.9	20.8	20.8	20.7	20.6	20.5	20.4	20.2
25	21.0	21.0	21.0	20.9	20.9	20.9	20.8	20.8	20.7	20.6	20.5
50	21.0	21.0	21.0	21.0	21.0	20.9	20.9	20.9	20.9	20.8	20.8
75	21.0	21.0	21.0	21.0	21.0	20.9	20.9	20.9	20.9	20.9	20.9
100	21.0	21.0	21.0	21.0	21.0	21.0	21.0	20.9	20.9	20.9	20.9

Table 1: oxygen concentration, %: effect of evaporation; 0.4 air changes/hour

Room volume m3	Volume of liquid nitrogen, litres										
	10	25	50	75	100	150	200	250	300	400	500
15	18.6	18.6	18.5	18.5	18.5	18.4	18.3	18.2	18.1	18.0	17.8
25	19.6	19.5	19.5	19.5	19.5	19.4	19.4	19.3	19.3	19.2	19.1
50	20.3	20.3	20.3	20.2	20.2	20.2	20.2	20.2	20.1	20.1	20.0
75	20.5	20.5	20.5	20.5	20.5	20.5	20.4	20.4	20.4	20.4	20.3
100	20.6	20.6	20.6	20.6	20.6	20.6	20.6	20.6	20.6	20.5	20.5

Table 2a: oxygen concentration, %:
effect of topping-up with 10litres LN + evaporation; 0.4 air changes/hour

Room volume m3	Volume of liquid nitrogen, litres										
	10	25	50	75	100	150	200	250	300	400	500
15	20.0	20.0	20.0	20.0	20.0	19.9	19.9	19.9	19.9	19.8	19.7
25	20.4	20.4	20.4	20.4	20.4	20.4	20.3	20.3	20.3	20.3	20.2
50	20.7	20.7	20.7	20.7	20.7	20.7	20.7	20.7	20.7	20.6	20.6
75	20.8	20.8	20.8	20.8	20.8	20.8	20.8	20.8	20.8	20.7	20.7
100	20.9	20.9	20.9	20.8	20.8	20.8	20.8	20.8	20.8	20.8	20.8

Table 2b: oxygen concentration, %:
effect of topping-up with 10litres LN + evaporation; 1 air change/hour

Room volume m3	Volume of liquid nitrogen spilled, litres						
	1	2	3	4	5	10	25
10	19.6	18.1	16.7	15.3	13.8	6.7	
25	20.4	19.9	19.3	18.7	18.1	15.3	
50	20.7	20.4	20.1	19.9	19.6	18.1	
75	20.8	20.6	20.4	20.2	20.0	19.1	16.2
100	20.9	20.7	20.6	20.4	20.3	19.6	17.4

Table 3: oxygen concentration, %: effect of spillage

APPENDIX B

TRANSPORT OF LIQUID NITROGEN IN LIFTS

The safe transport of cryogenic gases in lifts can be only be accomplished in lift 7 and lift 52. The procedure is similar for each lift, the essential features being:

- It is a two-person operation
- The lift is controlled by the use of keys from the Service Level
- Lift 52 serves floor 3 and below
- Lifts 7 has two separate key-operated services. One is a backup for lift 52 and serves floors 3 and below. The other service is for floors 9 and 10 only.
- Keys are issued to departments that are major users of cryogenic gasses.
- Keys are also available from the Medical School Porters Reception on the Lower Ground Floor (ext. 4240/4338 or bleep 603/108).
- The times when this operation can be carried out are : -
Monday – Friday - Before 8.30am; 10.30am – 12.00 noon; 2.30pm – 4.30pm; After 6.00pm
Saturday, Sunday & Bank Holidays - at any time

The lift is disabled from normal use but as each operation should only take approximately one minute the disruption should be minimal - provided the lift is not left in the “Special Service” mode while containers are being filled on the Loading Bay.

“Empty” cryogenic gas containers should be treated in the same way as full ones if there is any residual liquid present.

To send a full container from the Service floor to another floor

From the Service floor, call the lift in the normal way.

When the lift arrives, turn the “Special Service” keyswitch to the “on” position (red key).

N.B. Do not turn the Special Service keyswitch to the on position before the lift has arrived at the service floor. Doing so will prevent the lift from being called.

Load the Liquid Nitrogen container into the lift. **DO NOT ALLOW ANYONE TO ENTER THE LIFT AT THE SERVICE LEVEL.**

Send the lift to the desired floor, to be received by a colleague, by turning and holding the appropriate keyswitch (green key). The keyswitch must be held over until the lift doors have closed.

N.B. Do not press any button inside the lift as this will override the keyswitch and the lift will also stop at that floor.

Allow sufficient time for the container to be unloaded. Once the container has been unloaded the “Special Service” keyswitch can be returned to the “off” position to return the lift to normal operation.

A telephone (extension number 8862) has been provided by Lift 52 on the Service Floor in order to aid communication between Staff carrying out these operations. The extension number of the telephone in Lift 52 is 4392. The number of the telephone near to lift 7 is 4600.

To send a part-filled container to the Service floor from another floor:

The procedure is similar to that above:

1. The lift is called to the Service Level
2. The over-ride keys are used to send the empty lift to the desired floor
3. When loaded, the lift is called back to the Service Level by using the appropriate keyswitch
4. Once the lift has been unloaded, the lift is put back into normal service by removal of the special keys.

NB. Only containers that have no residual liquid present may be accompanied during transportation in a lift.

Please report any problems in implementing this procedure to either :-

Safety Office
RFH NHS Trust
ext. 8035

or

Mr.G.E.Goldsmith
Assistant Head of Administration
RF&UCMS
Royal Free Campus
e-mail gedgold@rfc.ucl.ac.uk.
ext. 4277

APPENDIX C – risk assessment proforma

Question	Yes / No	Comments
Storage		
Location of store (with room number)		
What is the volume of LN (litre)?		
What is the volume of room (m ³)		
Is there good natural ventilation?		
Is the air change rate in room >0.5 per hour?		
Is there an oxygen depletion alarm?		
If yes:		
• Is it audible/visible from outside?		
• Is a malfunction audible/visible from outside?		
• Is it checked periodically according to manufacturer's recommendations?		
Transport		
Which lift do you use?		
Use		
Location (eg lab name/room number, floor)		
Is there good natural ventilation?		
Is the ratio of room volume (m ³)/volume LN (litre) in room >15?		
Is the air change rate in room >0.5 per hour?		
Is there an oxygen depletion alarm?		
If yes:		
• is it audible/visible from outside?		
• is a malfunction audible/visible from outside?		
• is it checked periodically according to manufacturer's recommendations?		
PPE		
What PPE is provided to protect the following (give BS EN number where appropriate):		
Face		
Hands		
Arms		
Body		
Feet		
Training		
Have all staff been given training as necessary in the transport, use and disposal of LN?		

APPENDIX D - STANDARDS

risk assessment

1. A suitable and sufficient risk assessment shall be completed for all aspects of the use of liquid nitrogen.

ventilation in rooms

2. Wherever possible, LN should be stored in rooms having good natural ventilation.
3. Where this is not possible:
 - the room must have forced ventilation of at least 1 air change an hour.
 - the room must have an oxygen deficiency alarm installed inside the room.
 - the alarm signal must be audible/visible from outside the room.
 - alarm malfunctions must be visible/audible from outside the room.
 - the alarm must be checked periodically following the manufacturer's recommendations.

transport inside the building

4. The transport of LN dewars between floors is permitted only by means of lift 7 or lift 52.
5. People must not travel in a lift with a LN dewar containing LN.
6. The procedure described in Appendix B must be followed.

transport in road vehicles

7. The liquid nitrogen container must be in a separate compartment from the driver and passengers.
8. The container must be secured to prevent movement during travel
9. There must be a constant airflow into the passenger compartment of the vehicle
10. The driver must be made aware of the hazards associated with liquid nitrogen
11. The driver must be given written information containing details of:
 - Contact names and numbers at the hospital
 - What to do in the event of a spillage
 - What to do in the event of an incident
 - What to do in the event of contamination to himself.

training

12. Employees must be given adequate instruction and training on the hazards, precautions, and emergency procedures.

personal protective equipment

13. The personal protective equipment needed for the task shall be identified in the risk assessment
14. The personal protective equipment identified in the risk assessment shall be
 - provided
 - suitable for each person having to use it
 - be used properly at all relevant times
 - be adequately maintained.