

# Guidance for the safe operation of liquid nitrogen freezers for cryo-preservation.

Biological activity reduces as temperature decreases. At temperatures below about  $-135^{\circ}\text{C}$ , this activity effectively stops and therefore biological materials (e.g. cells, tissues, blood) can be stored at  $-135^{\circ}\text{C}$  or below without significant deterioration. This process is known as cryo-preservation. Commonly this is done by storing the samples in freezers containing nitrogen in its liquid phase ( $-196^{\circ}\text{C}$ ) or its cold vapour phase ( $-135^{\circ}\text{C}$  to  $-190^{\circ}\text{C}$ ).

This Safetygram addresses the safe use of liquid nitrogen cryo-preservation equipment. The hazards posed by this equipment are commonly due to:

- The expansion of liquid nitrogen (by a factor of about 700) as it evaporates. This can lead to a displacement of oxygen from the atmosphere in poorly ventilated areas, which can cause asphyxiation and ultimately may be fatal. Storage or transport of liquid nitrogen in unvented containers will rapidly lead to over-pressurisation and explosion.
- The extremely low temperature of liquid nitrogen which can burn exposed flesh. Many materials become brittle at liquid nitrogen temperatures so vessels and piping have to be constructed from a limited range of suitable materials.



## General Precautions

**Guard against oxygen deficiency.** There have been incidents where users of freezers have been exposed to potential harm when nitrogen has escaped from a cryogenic storage vessel and reduces the surrounding oxygen concentration to an unacceptably low level. Persons using or maintaining the freezing equipment need to be aware that although nitrogen itself is non-toxic, it can reduce the oxygen concentration of atmospheric air locally to levels that may become hazardous to health and potentially fatal. Atmospheric air contains about 21% oxygen, and reductions in this concentration are increasingly harmful, diminishing mental alertness such that a person may not recognize symptoms nor realize they are in danger. There may be no visual indication of an oxygen depleted atmosphere, but sometimes a white water vapour cloud may be present due to the cooling effect of the nitrogen. A vapour cloud usually indicates some oxygen depletion, but do not assume the absence of a cloud means there is a normal atmosphere.

Be aware of the first symptoms of oxygen deficiency – increased pulse and breathing rate, giddiness and loss of judgment. Air Products' Safetygram 17, Dangers of Oxygen-Deficient Atmospheres, gives more details on the dangers of oxygen-deficient atmospheres.

**Wear protective equipment.** Liquid nitrogen is extremely cold, and contact with it, or any part of the freezer that has recently been in contact with it, may cause immediate frostbite or cold contact burns to unprotected skin. For hand protection wear loose-fitting, thermally insulated gloves. Do not, even when wearing protective equipment, expose yourself to low temperatures for longer than is essential. Do not breathe the cold vapour phase (gas) as this can cause lung tissue damage. See also “Safetygram 7 title “Liquid Nitrogen” for more information on the hazards of liquid nitrogen.



**Use the correct equipment.** Because of the large amount of gas created by evaporation of liquid nitrogen, for storage and transport do not use anything except equipment specifically designed for the purpose. Unvented containers must not be used. Many materials become extremely brittle and likely to fracture at liquid nitrogen temperatures.

**Do not lean into the freezer**

The atmosphere inside the freezer is unsafe for breathing. There may be cold surfaces which one inadvertently contacts when leaning into or onto the freezer.

**Do not contaminate the freezer.**

Take care not to allow contamination into the freezer.

### Specific operational advice

People must not enter or occupy areas suspected of having reduced oxygen concentrations, specifically where the concentration is less than 19.5% oxygen. To minimise the possibility of this happening, the freezers should be installed in adequately ventilated rooms.

To ensure that the ventilation is adequate, a risk assessment of each installation is required. It is the responsibility of the facility operator to complete the assessment and implement its findings. Air Products can provide assistance with regard to this assessment. This risk assessment will normally require that:

- Room oxygen monitors be installed, usually with multiple sensors no more than 4m apart.
- The oxygen monitors are operational 24 hours per day. They should not be easily switched off (no plug or switch). Battery back-up is advisable.
- The oxygen monitors have an alarm level of 19.5% oxygen to notify personnel to evacuate the area.
- The nitrogen supply is interlocked to

the oxygen monitor alarms.

- There is an indication outside all entrance doors to the freezer room that the atmosphere inside the room is safe or unsafe.
- There is an air extraction system that ventilates all parts of the freezer room and any adjacent room that may have limited ventilation.
- Ideally, the air extraction system should be operational for 24 hours per day and should boost to a higher speed when the oxygen monitor is in warning or alarm state.
- Extracted air is extracted at low level and is replaced by fresh air and not re-circulated air. (Most air-conditioning systems do not supply sufficient fresh air.)
- Operating personnel undergo initial and ongoing training.
- If a freezer is to be disconnected from a permanently installed supply, line connected to a LIN

storage tank, the connection point on the pipeline must be capped to minimise the chance of a nitrogen leak

Operation and maintenance of oxygen monitors is the responsibility of the facility operator of the cryo-preservation installation. It is important to keep oxygen monitors calibrated and maintained in good working order according to manufacturer's specifications. Personal oxygen monitors may be used to provide additional protection by monitoring in the vicinity of the worker's breathing zone.

Thermal relief valves must be installed between valves to prevent trapped liquid from over-pressurizing piping. These relief valves must be set higher than that of the pressure relief system at the supply tank and should be piped either outside or to a safe location.

When opening freezers for loading or removal of samples, do not put your face close to the entrance of the freezer. The oxygen level is likely to be low and the cold gas can injure your lungs.

A comprehensive procedure shall be developed detailing the method that should be used when operating the equipment. Emergency preparedness shall be a major part of the instruction and only operators trained in the dangers of asphyxiants should be allowed to carry out these tasks.

Avoid unnecessary movement of individual freezers if they are connected to the nitrogen supply. A failure of a flexible pipeline will cause a major nitrogen leak. If this happens, evacuate the room immediately and attempt to shut off the nitrogen supply from outside if safe to do so. Makes sure that at all times there is an unrestricted exit route from the freezer room.

## Filling warm vessels

When liquid nitrogen evaporates it produces about 700 times its volume of gas. So when warm vessels are being filled there will be large volumes of gaseous nitrogen created. If warm vessels are to be filled, a further risk assessment is required which should consider:

- Restriction on the flow of liquid nitrogen.
- Possible additional air extraction.
- Pause the filling at intervals to allow oxygen levels to recover.
- Personal protective equipment for the operator (gloves and full face mask as a minimum).
- Personal oxygen monitor.
- Work instructions for the operator.

This Safetygram does not cover the design of the freezers themselves but, in Air Products' opinion, the freezer should have two independent circuits to monitor liquid nitrogen level in the freezer so that over-filling is highly unlikely. Do not alter any temperature/level alarm settings without con-

sulting the manufacturer. Air Products also recommends an automatic safety shut-off valve which cuts off the nitrogen supply to all freezers in the event of an emergency. A typical emergency will be a low oxygen alarm, a deliberate pressing of an emergency button or a control circuit failure.

Do not change any component on the freezer without consulting the manufacturer. Apart from potential safety risks, it may affect CE certification. Also be aware that any loss of insulation will significantly increase liquid nitrogen use and/or result in increased temperatures in the freezer.

Ensure all relevant personnel are trained in the hazards of liquid nitrogen. Safetygram 7, Liquid Nitrogen, and Safetygram 17, Dangers of Oxygen-Deficient Atmospheres, provide more information <http://www.airproducts.com/safetygrams>

For Safety Data Sheets, visit <https://www.apdirect.airproducts.com/msds/>

For liquid nitrogen on-line safety training visit <http://www.gassafeinteractive.co.uk/airproducts/>



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