

## Cryosafety in the Embryology Laboratory

Following the recent death of a police officer investigating a leakage of liquid nitrogen (LN<sub>2</sub>) at Xytex Corporation in Atlanta, Georgia, USA (<http://www.dailymail.co.uk/news/article-4195852/Georgia-sheriffs-deputy-dies-inhaling-liquid-nitrogen.html>), cryosafety has become a topical issue. Despite such evident dangers, an excellent survey of the risks associated with liquid nitrogen, conducted by Mathew Tomlinson and David Morroll at a joint meeting of the Association of Clinical Embryologists and Association of Irish Clinical Embryologists in 2006, reported in Human Fertility (<https://www.ncbi.nlm.nih.gov/pubmed/18320438>), concluded that incidents involving liquid nitrogen were more frequent than expected, and that training and awareness of risks associated with cryostores was generally lacking. Therefore, it seems pertinent and timely to revisit this issue.

An important starting point in liquid nitrogen cryosafety is the design of the cryostore where most handling of liquid nitrogen will occur. In this respect, it is recommended that a safety audit be conducted by appropriately qualified personnel prior to establishing a cryostorage facility. Because liquid nitrogen expands rapidly as it boils at -196°C, the liquid:gas expansion ratio being 1:790, it can dramatically reduce the concentration of oxygen in the atmosphere within an enclosed or poorly ventilated space, leading to asphyxiation without any warning symptoms. Therefore, it is essential to install an oxygen monitor within a cryostore to provide early visible and audible warning to allow staff adequate time in which to evacuate the room, in the event that nitrogen displaces oxygen to significantly lower levels than those normally present within the atmosphere. It is also good occupational health and safety (OHS) practice to link the low oxygen monitor to extraction fan-assisted ventilation which is automatically ramped up once the low oxygen alarm has been triggered in order to rapidly remove the nitrogen and replenish oxygen to atmospheric levels.

Due to the very low temperatures at which liquid nitrogen and its vapour exist, they can potentially inflict burns, or even frostbite, if mishandled. Therefore, good OHS practice mandates that appropriate and sufficient personal protection equipment (PPE) must be provided for staff working within a cryostore. Cryogenic gloves and safety glasses are typically available within most laboratories but PPE should also include a face shield, protective apron, fully enclosed shoes and long sleeved clothing. It is necessary to instruct and educate staff in the use of PPE and the variety of hazards associated with handling and storing liquid nitrogen. Instructions should include written standard operating procedures (SOPs) and prominently displayed notices within the cryostore, including evacuation maps. Education in liquid nitrogen cryosafety should be an integral component of the facility's staff orientation and may be regularly supplemented with courses provided by suitably qualified external organisations such as ACE (<https://www.embryologists.org.uk/calendar/event/view?id=36>) and Gas Safe Ltd (<https://www.gassafeconsultants.co.uk/gas-safety-training/using-liquid-nitrogen-safely/>). In addition to burns and frostbite, the typical hazards that staff may be exposed to include eye injury and infection resulting from explosion of straws or cryovials due to rapidly expanding nitrogen upon warming of any liquid nitrogen that may have seeped into them during cryostorage. Hence, it is important to only use straws and cryovials that have been certified by their manufacturer for storage in liquid nitrogen.

In many countries, provision of a licence to provide a clinical embryology service is contingent upon having in place appropriate monitoring, alarms, safety equipment, policies and procedures necessary to deal effectively and safely with unexpected leaks or spills of liquid nitrogen from bulk liquid nitrogen supply tanks or storage dewars. For example, the codes of practice of the Human Fertilisation & Embryology Authority (<http://www.hfea.gov.uk/code.html>) in the UK and the Reproductive Technology Accreditation Committee (<http://www.fertilitysociety.com.au/rtac/accreditation-documents/>) in Australia both cover such requirements. From a European perspective, the European Society of Human Reproduction guidelines for good practice (<https://www.eshre.eu/Guidelines-and-Legal.aspx>) cover cryosafety in embryology laboratories in considerable detail. However, not all countries where cryostorage of gametes and embryos is performed have in place a similar regulatory framework for protection of staff working within embryology laboratories. Under such circumstances, it is very important that cryostore working areas remain well ventilated when in use and that staff do not work alone or investigate any low oxygen alarms on their own. These principles should be clearly stated within the facility's relevant SOPs and are an essential component of any organisation's quality management system.

In an ideal world, nobody would suffer any injuries from working with liquid nitrogen, but in the real world tragedies such as those that occurred recently at Xytex can happen. Therefore, it is incumbent on us all to follow best practice when handling liquid nitrogen so as to minimize the risks to ourselves and our colleagues working within embryology laboratories.

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