/BIO Q&A

The Culture of Cryopreservation

Supercooling, seeding, and subzero safety

COMPILED AND EDITED BY JOHN HODGSON

This month's BIOQ&A endeavors to bring a thaw to the frosty world of cryogenics and preservation with contributions from ***Ian Blackham*** *at Planer Products (Sunbury, U.K.) and* ***Jim Covault****, from the CryoMed Division of Forma Scientific (Marietta, OH).*

Q. *What is the optimum temperature for long-term cryopreservation storage?*

A. While many cultures are stored at -70°C for months or even years, metabolic activity does not cease; it is only slowed down. Storage at temperatures below the "glass transition of water," or -120-130°C, is where, it is said, biological time is stopped and cultures may be stored for a millenium. (JC)

Q. *How does freezing actually occur?*

A. Ice formation begins at a nucleation site that can be a randomly occurring cluster of molecules of the liquid phase. Removal of the latent heat released during the phase change favors cluster growth and is promoted by lower temperatures in the surrounding environment. The nucleated ice crystal forms into an ice front expanding through the liquid until solidification is complete. (IB)

Q. *What are the advantages/disadvatages of choosing liquid or vapor phase liquid nitrogen storage?*

A. Liquid phase storage offers a uniform temperature of -196°C. The problems are that cryovials, used for sample storage have a tendency to leak, allowing liquid nitrogen to enter the sample and possibly permitting samples to cross-contaminate each other. When a vial is retrieved from storage, any trapped liquid nitrogen reverts to gas—a manyfold expansion in volume that could cause an explosion, unless the gas is allowed to escape.

Vapor phase storage does not offer a uniform temperature for the storage of samples. Instead, there is a gradient of temperature. It is not unusual, in a wide-mouth dewar, to have a 75-100°C gradient from the temperature of the liquid to the temperature under the lid of the container. The extent of the gradient can be reduced by raising the liquid level or its slope can be reduced by adding conductive materials, such as aluminum, to redistribute the colder temperature to the top. However, the gradient can only be eliminated by use of liquid phase. (JC)

Q. *What happens if I place my biological sample directly into liquid nitrogen?*

A. Rates of freezing that are too fast or slow contribute to cell destructions by causing intracellular ice crystal formation and/or cellular dehydration while the cell attempts to maintain its osmotic balance during the freezing process. Experience has shown that a rate of 1-3°C per minute (with a temperature compensation for the latent release of heat) will provide improved post-freezing viability. (JC)

Cells can be damaged if cooled too quickly or too slowly. The optimum rate of cooling varies with the cell type. Controlled rate freezers have been developed by several manufacturers to ensure reproducibility and accuracy in routine cryopreservation of cells. These provide a high degree of sample safety onco the optimum conditions have been established. After freezing, cells are commonly stored long-term below -120°C, ideally in liquid nitrogen. (IB)

Q. *What are the safety concerns when using liquid nitrogen?*

A. Liquid nitrogen is a cryogen with a boiling point of -196°C (-320°F). In handling it, common sense will go a long way. The following specific points should always be considered: (1) Wear protective clothing; (2) use only containers designed for low-temperature liquids; (3) do not seal or prevent liquid nitrogen from venting; (4) never use hollow rods as measurement sticks; (5) use liquid nitrogen only in well-ventilated arears; and (6) do not overfill containers.

There is another general point. Nitrogen gas is colorless, odorless, and tasteless. It cannot be detected by the human senses and will be breathed as if it were air. Breathing an atmosphere that contains less than 18% oxygen can cause dizziness and quickly lead to unconsciousness and death. (JC)

Q. *What is meant be "seeding" of samples?*

A. Seeding is a technique used to induce controlled crystallization in solutions that have already been cooled below freezing point. Samples can frequently be damaged by "supercooling" during freezing. Seeding can minimize this damage and is, therefore, often essential for cell survival. (IB)

Q. *What are the power requirements for long-term storage in liquid nitrogen?*

A. Power is not required for the safe storage of samples at -196°C. Power is only required to support any electronics associated with the storage unit—for autofilling, monitoring, and alarms, for instance. Power failures will only require that such operations are conducted manually and will not affect the storage of samples. (JC)

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