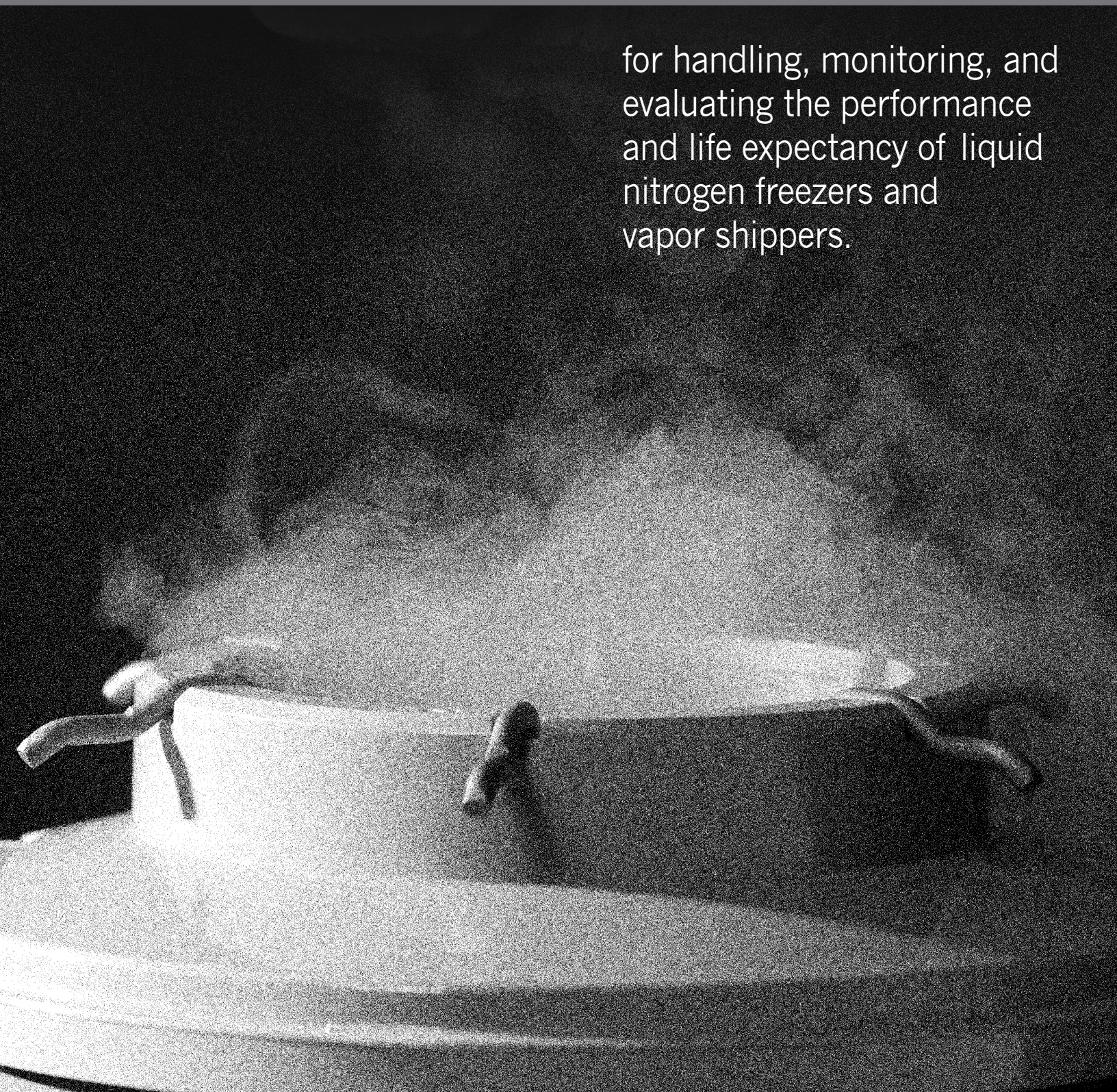
COLDSTASH

Recommended best practices and policies

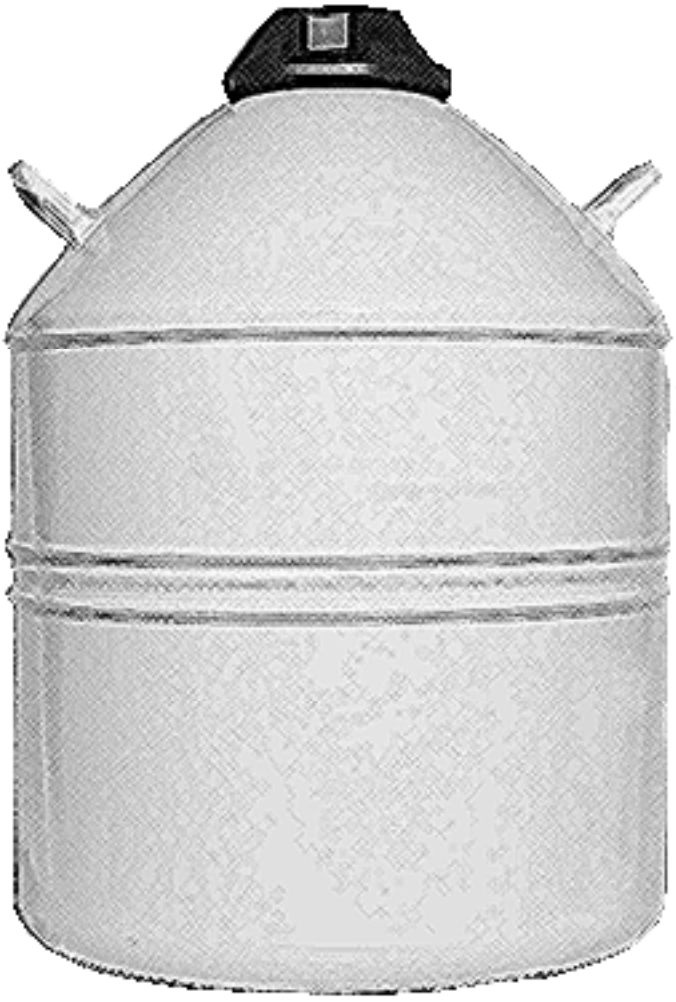
for handling, monitoring, and evaluating the performance and life expectancy of liquid nitrogen freezers and vapor shippers.



INTRODUCTION

To mitigate unexpected failure and/or sample loss, one should never assume a liquid nitrogen (LN_2) dewar will efficiently maintain adequate cryogenic temperatures. Many factors, including techniques in monitoring liquid level and temperature, usage, handling, and having an overall “respect” for the capabilities and limits of a dewar need to be recognized.

LN_2 dewars, when selected, validated, put into use, monitored, periodically evaluated, and operated properly can provide many years of dependable and predictable cryogenic performance, generating ultra-cold temperatures that many biological samples require. Just because a dewar contains LN_2, however, does not mean that it will be cold enough for the survival of valuable and often irreplaceable samples.



BEST PRACTICES AND POLICIES

Establishing best practices and policies for handling, monitoring, and evaluating the performance and life expectancy of a LN_2 dewar begins with understanding the manufacturer's specifications and capacity of selected unit. Once understood, one should a) define and monitor expected performance, b) establish regular testing and dewar evaluation, and c) mitigate risk.

MANUFACTURERS SPECIFICATIONS

It is important to understand that the desired sample storage temperature is dependent upon the actual level of LN_2 in the dewar; appropriate level will vary across dewar make/model. Beyond level of LN_2, it is necessary to understand and record the manufacturer's specifications prior to establishing policies for a specific dewar. Examples of such essential specifications include:

- Date of manufacture and initial operation
- LN_2 capacity (L)
- Neck opening (mm or inches)
- Static loss rate (liters per day without opening lid/neck cork)
- Static holding time (holding time of undisturbed liquid level in days)
- Usable internal height (mm or inches available for sample storage)
- Sample inventory capacity (number of canisters, vials, straws, etc)
- Weight empty/weight full (used to perform static loss validation)
- Vapor Phase or Liquid Phase performance data

DEFINE AND MONITOR EXPECTED PERFORMANCE

Handling and Use:

Frequent or prolonged opening of a dewar may lead to increased static loss rate of LN_2, exposing existing samples to subtle but potentially severe warming events that may endanger sample viability and use. To prevent such an event, one should consider the following prior to establishing lab policies:

- If the dewar will be used for long term (static) storage of samples (rarely opened for sample access), or for active use (frequent removal and adding)
- Will the dewar be actively opened and accessed (daily/weekly) to retrieve and/or add samples
- Frequently removing and adding samples (depending on how the samples are inventoried or apportioned) may subject existing/stored samples to a transient warming event
- May any of the inventory be backed up or "split" between two separate dewars
- How frequently should the dewar be filled or "topped off" with LN_2
- Excessive movement or inadvertent damage upon moving the dewar or overfilling the dewar above the usable storage area can affect liquid level and temperature performance
- Proper filling and avoidance of excessive "splashing" or prolonged contact of LN_2 with the neck tube, cork area and vacuum port/valve

METHODS OF DAILY MONITORING

Significant temperature fluctuations or gradients are directly related to the position of the temperature sensor and the level of LN_2. The temperature gradient created by the distance between the actual liquid level and the temperature sensor can make the difference between an acceptable and unacceptable temperature, especially in temperature critical samples that may be stored for longer periods of time such as those stored for IVF and cell therapy. In addition to proper monitoring, regular movement or disturbance of samples intended for long term storage, frequent lid openings, and drastic liquid level fluctuations should be avoided to prevent potential thermal warming events.

Temperature Monitoring:

Temperature monitoring is performed "real-time," with the temperature sensor located at the warmest part of the dewar, typically just below the neck opening. Temperature monitoring is dependent upon a calibrated temperature sensor or thermocouple/thermistor connected to an external digital monitor. The monitor, in addition to providing real-time temperature reads, may trigger an alarm (locally or remotely) if the temperature rises above the desired and acceptable temperature range.

The temperature sensor should be securely positioned and affixed furthest away from the liquid level and at the highest sample location (bottom of the neck cork). The sensor should not be subjected to any inadvertent movement during the active use of removing and replacing the lid of the dewar or canisters and racks.

Always assume that there can be a significant difference in temperature (temperature gradient) from the LN_2 level (-196^0 C) to the location of the temperature sensor nearest the samples.

Liquid Level Monitoring

Digital liquid level measuring and monitoring is achieved through a liquid level sensor and monitor with local and remote alarm capability (if available). A liquid level monitor sensor should be positioned at a distance from the neck opening that, if the LN_2 falls below this position, will no longer provide acceptable sample temperature at the warmest part of the dewar.

MANUAL LIQUID LEVEL MONITORING SHOULD BE PERFORMED DAILY USING THE DIP-STICK METHOD.

ESTABLISH REGULAR TESTING AND DEWAR EVALUATION

Standard testing policies

Daily, weekly, monthly and bi-annual inspection/verification of temperature and liquid level sensors, alarms, sensor locations, and dip stick measurements should be recorded.

Temperature and liquid level alarms (local and remote) should be verified (triggered) and tested weekly.

The general physical condition and age of dewars, signs of ice, condensation, frost, condition of lid/cork, and any changes in the exterior condition should be documented.

A static loss rate test should be performed bi-annually or annually depending on the age of the unit. This test will determine any variation from the manufacturers original static loss rate. This will require a 7-day period of daily weighing and measuring liquid level in a dewar that is not accessed, filled, "topped off," or opened during this time. This may require transferring all sample inventory into the back-up dewar while this is being performed. Older dewars that exceed manufacturer's vacuum warranty (2-5 years) should be tested more frequently.

Performance Evaluation and life expectancy

The life expectancy and indications that a LN_2 dewar is performing within specifications can be determined by liquid loss rates, physical condition, and frequency of needing to fill.

Review all daily, weekly, monthly, and bi-annual liquid level and static loss rates that do not meet or are not in reasonable range of the original manufacturers specifications.

Document and report any physical signs such as "sweating", buildup of frost or ice on the dewar, and if detected, plan on immediately moving all samples to a back-up dewar until a tank evaluation can be performed.

Increases in the frequency of needing to fill a LN_2 dewar should be validated and tested more frequently. A vacuum failure does not necessarily occur instantaneously or rapidly.

Policies of mandatory replacements of LN_2 dewars after a period (say 10 years or more) may not necessarily be warranted, but units of this age should require more frequent static loss testing, along with temperature and liquid level review history.

MITIGATING RISK

One should always plan for a LN_2 dewar to lose vacuum and ''fail'' at any time without significant notice. Therefore, it is advisable to have sufficient, redundant LN_2 dewars (onsite or offsite) that have adequate sample inventory space.

Although LN_2 dewars depend on a reliable source of either automatic or manual gravity fed LN_2 (low pressure 22psi) delivery to maintain temperature and adequate liquid level, it is essential to understand that the integrity and performance of the vacuum insulation is paramount to the rate in which the LN_2 evaporates. Increased evaporation rates, as stated above, are also caused by frequent and prolonged opening of the lid, which may subject sample inventory to warming events.

The vacuum insulation of a LN_2 dewar is not a permanent insulation. It will degrade, albeit very slowly under normal conditions and proper use. The expected life of the vacuum insulation may depend upon age or years of use, types of use/abuse, and whether it has been subjected to cycles of complete warming ("going hot") and subsequent refilling ("recharging"). The vacuum space of a LN_2 dewar, (similar to a thermos bottle) is sealed by joints that may expand and contract under sudden temperature fluctuations.

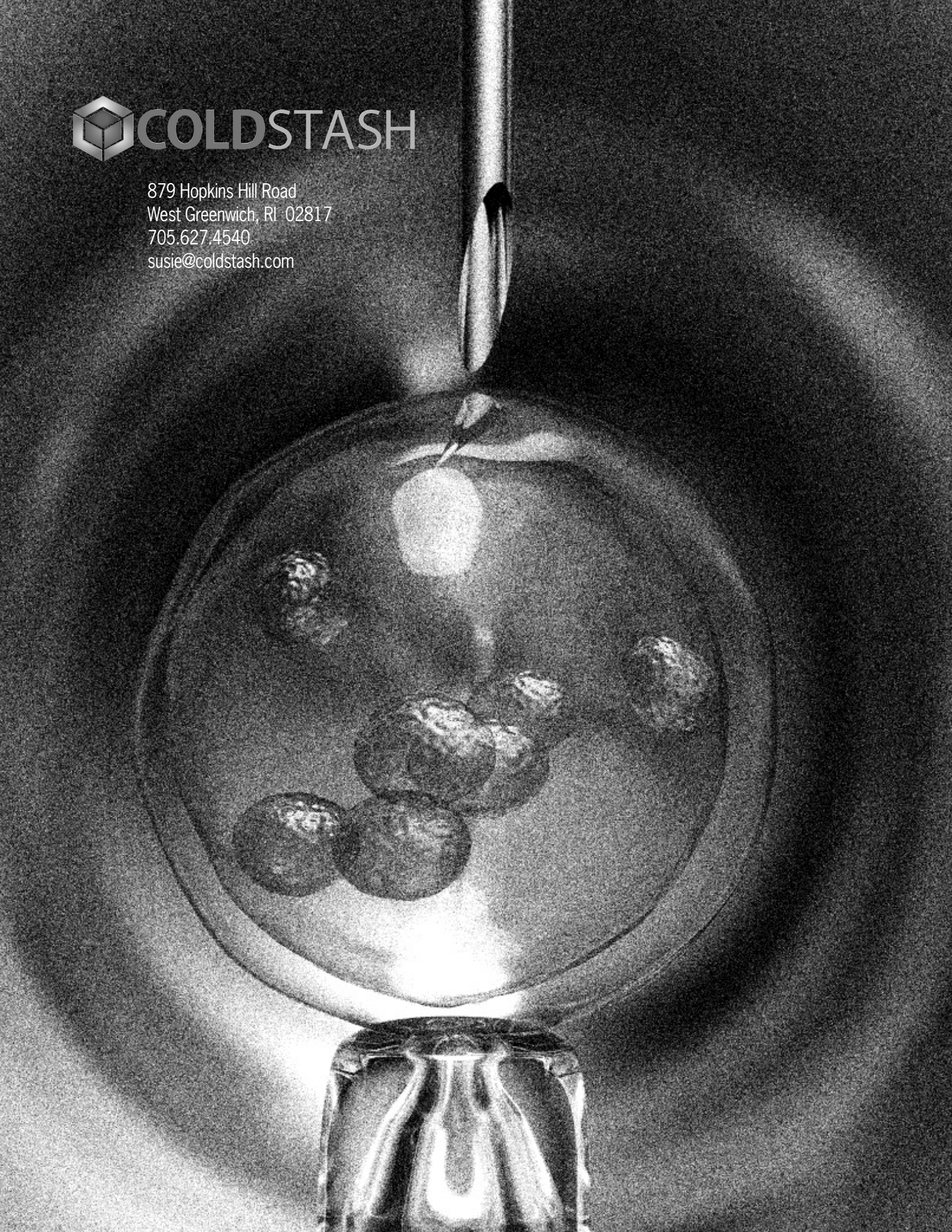
To mitigate the risk of rapid temperature rise and sample loss, it is recommended to have a validated backup dewar (or multiple dewars if necessary) that is empty (no samples) and cold ("charged"), with capacity to receive and hold sample inventory from a failing dewar. It is advisable to divide and store a client's samples or multiple sample copies into separate LN_2 dewars, in case of one failing. It is not recommended to "put all your eggs in one basket."

CONCLUSION

To create a robust, successful program, one should:

- Adhere to manufacturer's specifications
- Practice safe and efficient handling of dewars and samples
- Set up proper monitoring (temperature sensor, liquid level sensor, manual dip-stick)
- Regularly test and document all monitoring systems and dewars, including visual inspections
- Mitigate risk by keeping a charged backup dewar and dividing clients' samples

It is important to understand that vacuum insulation will eventually weaken in all dewars. Though most will last well past their warrantees, with many existing dewars still in use after 25+ years, this alone is not a guarantee that samples are being adequately stored. By following the guidelines outlined above, the custody of your biological specimens will be secured without degradation.


COLDSTASH
879 Hopkins Hill Road
West Greenwich, RI 02817
705.627.4540
susie@coldstash.com