Cryostorage of reproductive tissues in the in vitro fertilization laboratory: a committee opinion

Practice Committees of the American Society for Reproductive Medicine, Society for Reproductive Biologists and Technologists, and Society for Assisted Reproductive Technology

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This document is designed to assist in vitro fertility clinics in the management of cryopreserved reproductive tissues stored in cryogenic storage (cryostorage) tanks, based upon scientific principles and laboratory experience related to best practice for safe and reliable storage of cryopreserved reproductive tissue. Embryology and andrology laboratories provide storage of often irreplaceable reproductive tissues such as oocytes, embryos, sperm, and ovarian and testicular tissues, including tissues from cancer patients. All of these reproductive tissues must be maintained under stringent conditions. (Fertil Steril® 2020;■:■–■. ©2020 by American Society for Reproductive Medicine.)

Cryostorage tanks are cylindrical stainless-steel or aluminum containers with multilayered insulated sides. The vacuum between the insulated layers prevents boiling and minimizes loss of liquid nitrogen (LN_2), allowing the tank to sustain LN_2 at a nearly steady rate. When submerged in LN_2, cryopreserved samples are securely maintained at a temperature of $-196^{o}C$.

Requirements and minimum standards for cryostorage management have been published by the College of American Pathologists (CAP), which accredits the majority of in vitro fertilization (IVF) laboratories nationally, and by The Joint Commission (TJC). In addition to required policies and protocols, this document outlines the best practices and practice preferences around the cryostorage of reproductive tissues. Each clinic should develop and adhere to its own rigorous policies for the management and monitoring of cryostorage tanks and LN_2 reserves (1–6).

MINIMUM STANDARDS/ REQUIREMENTS

The requirements and minimal standards regarding critical cryostorage management for IVF laboratories have been published by CAP (Table 1). The majority of IVF programs in the United States are members of the Society for Assisted Reproductive Technology (SART) and are required by SART to have an embryology laboratory that is accredited by either CAP or TJC to ensure that they are following this guidance. However, IVF clinics that are not SART members should also refer to these minimum requirements for best practices.

Accredited embryology and andrology laboratories that provide cryostorage of reproductive tissues are required to check tanks three times per week and/or have continuous monitoring via a level probe (these manual and electronic verifications may be complementary and not mutually exclusive); have continuous alarms that are tested at least quarterly; have enough LN_2 supply for emergencies; provide adequate personal protective equipment such as gloves, shield, goggles, and shielding of skin; store tanks in a well-ventilated storage area; ensure appropriate training of personnel; and have appropriate safety data sheets and display signage. Each program must develop its own policies and procedures that, at a minimum, meet these requirements and standards.

BEST PRACTICES/PRACTICE PREFERENCES

Access to LN_2 Supply Tanks and Cryostorage Tanks

Access to cryostorage tanks and LN_2 supply tanks should be restricted only to authorized personnel and trained laboratory staff. If a cryostorage tank storage room is utilized, a secure locking mechanism should be employed to restrict entrance. Cryostorage tanks may also be locked by combination or key lock to further limit access. Cryostorage tanks ideally should be housed in an area where they are visible to the laboratory staff, both for monitoring purposes as well as safety of the laboratory personnel. This could be in an

Received June 8, 2020; accepted June 9, 2020.
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Fertility and Sterility® Vol. ■, No. ■, ■ 2020 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2020.06.019

TABLE 1

Requirements and minimal standards for cryostorage according to the College of American Pathologists, September 17, 2019 (6).

Checklist number question	Evidence of compliance
Reproductive Checklist RLM.03940 The laboratory has a written procedure to monitor and maintain adequate liquid nitrogen (LN_2) levels and temperatures for all critical storage containers. The monitoring process must ensure that the level of LN_2 is never below the established minimum level. Storage tanks must be visually inspected (sweating, cracks, rusting) and the level of LN_2 measured, with records of these checks at least three times per week. Alternatively, tanks must be continuously monitored with an LN_2 level alarm or a weight-based alarm system.	Written procedure for monitoring LN_2 levels AND Records of visual inspection and measurement of LN_2 levels at defined frequency OR Records of continuous monitoring of LN_2 levels or weight
Reproductive Checklist RLM.03944 Adequate LN_2 supplies are maintained onsite.	The laboratory must have sufficient LN_2 supply to fill a spare storage vessel and/or to allow for freezing of specimens in an emergency
Reproductive Checklist RLM.03950 Alarms are monitored 24 hours/day (either remote or in the laboratory) and there is a written procedure for responding to alarms. The laboratory must be able to demonstrate how the system works and the process to ensure timely response to both audible and remote alarms. Alarm systems must be checked at least quarterly. Personnel responsible for responding to alarms must be trained to follow written procedures to correct the problem or take alternative measures.	Written procedure for monitoring alarms AND Records of response to the alarm
Laboratory General Checklist GEN.77500 Adequate policies, procedures, and practices are in place for the use of LN_2 and dry ice.	Practices for the safe handling of LN_2 and dry ice include: 1. The mandatory use of appropriate gloves, shielding of all skin, and the use of a face shield or safety goggles when decanting or entering an open container of LN_2 2. The mandatory use of insulated gloves, dry ice tongs or scoop, and safety goggles/glasses when handling dry ice 3. Storage and use of all containers of LN_2 and dry ice only in well-ventilated areas. Do not use or store dry ice or LN_2 in confined areas, walk-in refrigerators, environmental chambers, or rooms without ventilation. An LN_2 or CO_2 leak in such an area could cause an oxygen-deficient atmosphere. 4. Availability of a Safety Data Sheet 5. Training on the safe handling of LN_2 and dry ice 6. Signage displayed in areas where LN_2 is used and/or stored.

Note: CO_2 = carbon dioxide; GEN = laboratory general checklist; LN_2 = liquid nitrogen; RLM = reproductive laboratory checklist.

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anteroom that is visible behind a window, or a well-trafficked area of the laboratory. Tank storage rooms that are secluded or isolated from the daily laboratory activities pose an increased likelihood for issues not being detected because they are not readily visible at all times to the laboratory staff. In laboratories that preclude immediate proximity of tanks to well-trafficked areas, tank storage rooms should be checked regularly. Consideration should be given to having routes of safe delivery and removal of LN_2 supply tanks. Adequate ventilation and potential future storage needs should also be considered when determining an appropriate storage location.

Manufacturer's Recommendations

Manufacturers of cryostorage tanks have recommended procedures, which should be completed prior to the introduction and placement of new tanks into use. These recommendations vary based on the manufacturer and the type and size of the cryostorage tank. Cryostorage tanks are available as manual-fill traditional vessels and as large-capacity bulk storage units which employ an autofill function from secondary nitrogen storage tanks. Manufacturers offer time-limited vacuum warranties, but tank lifespan is difficult to estimate. In the absence of manufacturers' recommendations for the lifespan of tanks, follow an established protocol that includes visual inspection of tanks and assessment of tank integrity, that may include determining the presence of condensation or water pooling, frost, or ice, and cracks about the neck.

Newly acquired storage tanks and previously unused or empty storage tanks should receive initial LN_2 filling and functional assessment, according to the manufacturer's recommendation, prior to being placed into service. Functional assessment of the tanks should take a minimum of 7 days, but 30 days of assessment is recommended.

For older tanks, reliability within acceptable quality control limits can be validated through maintenance and

monitoring. While older tanks may be used with no issues, the clinic may be assuming additional liability if these tanks should fail. Each laboratory should make their own determination and have their own written protocol outlining their procedure for retiring cryostorage tanks.

Liquid vs. Vapor Cryostorage

Cryopreserved specimens may be stored in either the liquid or vapor phase of nitrogen. When samples are submerged in the liquid phase, the temperature remains stable and cannot rise abruptly. This allows personnel who respond to an emergency to have more time to rescue and relocate samples from the failing tank before the temperature rises catastrophically. Reproductive tissues are most commonly stored in the liquid phase of nitrogen.

Cryostorage in the vapor phase of nitrogen is also feasible. The vapor in which samples are stored is typically at -150^{o}C and lower. Vapor storage is commonly used for the storage of virus-positive patient samples where theoretically the risk of transmission of the virus is negated by the presence of vapor rather than of liquid. Vapor-phase nitrogen is also utilized in shipper tanks and the large bulk tanks in use at long-term storage repositories. Often, these large bulk vapor tanks are linked to an auto-fill nitrogen supply tank. Vapor storage tanks with an auto-fill function should be continuously monitored, as a catastrophic failure of the system will lead to a rapid rise in temperatures and insufficient time for personnel to respond before samples are compromised. Due to the increased potential of catastrophic failure in vapor phase nitrogen storage, careful analysis must be performed when deciding between liquid and vapor phase storage.

Quality Control of Cryostorage Tanks

Laboratories should create and strictly follow their own protocols and best practices for monitoring and quality control of their cryostorage tanks, following the guidelines of the CAP or TJC. Each laboratory should also determine its own critical storage level of LN_2 for each of their cryostorage tanks. Per CAP, the level of LN_2 must either be measured three (3) times per week, in inches or centimeters, or there must be a continuous monitoring system for LN_2 levels. The ability and length of time a cryostorage tank will hold a stable measure of LN_2 is determined by the type and size of tanks, as well as the integrity of the vacuum seal. In addition to LN_2 measures, monitoring of cryostorage tanks should include frequent visual and physical inspection. Observations such as condensation or water pooling, frost or ice, cracks about the neck and welded seams, or damage to the outer layer of the tank could be indicators of a vacuum failure and should be promptly addressed.

Reserves of LN_2 should be readily available to fill cryostorage tanks as needed, either physically or by autofill systems. Autofill tanks should also be monitored to ensure that they are well connected to an LN_2 reserve tank of the correct outlet pressure, as cryostorage tank failures have occurred when this precaution was not observed. Quality control documentation of both the tank's liquid level and the replenishment of LN_2, manually and by autofill, should be reviewed on a regular basis. Any tank that exhibits a loss of LN_2 through evaporation that is faster than expected should have its cryopreserved samples transferred to another tank, and the tank replaced. For this reason, it is recommended to have a spare tank, or sufficient space available in other validated cryostorage tanks to accommodate the samples from the failed tank. Any tank kept as a backup for emergency preparedness must be monitored and managed using the same protocol as tanks in active use by the laboratory. Despite adherence to all quality control and maintenance recommendations, any cryostorage tank can experience a catastrophic failure that could result in the loss of cryopreserved samples, and patients should be informed of this potential risk, however remote.

Oxygen Monitor

A unique risk to laboratory personnel associated with maintaining cryostorage tanks on site is the accidental creation of a hypoxic environment resulting from off-gassing of LN_2 from these tanks. For this reason, an oxygen monitor should be mounted in any area where LN_2 is decanted and where LN_2 tanks are stored. The monitor may be plug-in or hardwired and should be mounted in the breathing zone 4 to 6 feet from the ground, in order to quickly measure critical loss of oxygen in the room. The oxygen monitor should have both audible and visual alarms and the room should not be entered when the alarm is sounding. For the safety of the laboratory staff and others, routine testing should be performed on the oxygen monitor to ensure proper working order.

Cryostorage Tank Alarms

All storage tanks must be electronically monitored with probes that will sense a rise in temperature and/or a decrease in LN_2 levels. These monitoring systems should be associated with a remote alarm system that will immediately notify laboratory personnel when critical values are reached. Once a critical value is identified, the alarm system will notify laboratory personnel with an alarm in the laboratory as well as text messages and/or phone calls. All laboratories should have a clearly written response plan and phone tree for all alarms during both working and non-working hours.

Monitoring probes should be located in the tank so that an alarm trigger will provide sufficient time for personnel to respond before samples are compromised. Level probes suspended in LN_2 are recommended over temperature probes suspended in vapor, because laboratories that monitor only temperature may have a delayed and shortened response time if there is a vacuum failure; tanks with intact high-pressure vacuums can maintain adequate temperatures even with extremely low LN_2 levels.

Alarm systems should be able to operate in cases of power failure by being plugged into a backup generator or an uninterruptible power supply battery, or should be cloud based. The monitoring system should be tested on a frequent basis: at a minimum, quarterly.

Inventory of Storage Tank

Storage tank inventories are usually performed only when needed rather than on a regularly scheduled basis. Annual physical inventories are not routine since their benefit may be outweighed by the risk of inadvertent compromise of cryopreserved samples due to the fragile nature of these samples, especially vitrified embryos and oocytes. When a full cryostorage tank inventory is needed due to suspected loss of samples or discrepancies between the cryostorage records and the samples in the tank, the inventory should be conducted by laboratory personnel trained in handling cryopreserved tissue and a witness according to each program's policies and procedures.

Typically, when conducting an inventory, a canister of patient cryo-canes is temporarily relocated one at a time to an LN_2 bath. Vitrified embryos thaw extremely rapidly and can be damaged or even destroyed if warmed above the temperature required to maintain water in a vitrified state ($T_g = -120^{\circ}C$). It is therefore critical that labeling of the cryo-cane is legible while submerged under LN_2. Once the two unique patient identifiers are visible, the embryologist reads aloud the identifiers for the witness to hear and quickly counts the number of straws/vials on the cryo-cane. The witness then performs "active witnessing" by also visualizing the patient identifiers on the cane and verbalizing them aloud. Only if there is a need to reconcile the contents of the tank with what is recorded in the current tank inventory list should the straws be examined individually under LN_2. Once the inventory is accurate and complete, the inventory list should be updated and stored both electronically and in hard copy.

Sources of Errors and Failures

A number of sources of errors and failures, both human and mechanical, have been identified related to cryostorage in embryology and andrology laboratories. Table 2 summarizes the major categories and common issues that have occurred. Policies and protocols should be in place in every laboratory to ensure that, to the extent possible, these errors and failures are minimized.

TABLE 2

Known sources of errors and failures.

Equipment
Cryostorage tank failure due to breach of vacuum seal
Auto-fill malfunction on bulk storage tanks due to solenoid malfunction
Malfunction on the level sensor/float of an auto-fill tank
Alarm malfunction due to power outages or unrecognized issues due to failure of testing
Materials
Label fails and falls off carrier
Loss or damage of samples and/or carriers
Misuse of carriers or labels due to seal, bending, breaking, incorrect racking system, mixing of patients on same cane, incorrect materials for use in liquid nitrogen
Human error
Mislabeling or inadequate labeling of samples
Thawing sample(s) of the incorrect patient due to identifier issues
Inadvertent sample warming
Lack of quality control including liquid nitrogen filling schedule
Inadequate inventory records
Incorrect measurement of liquid nitrogen levels in tank
Failure to follow established protocols
Natural disasters
Fire, flood, earthquake, hurricane, tornado, etc.
Shipped sample errors
Inability to identify patient label
Inability to locate embryos
Number of embryos from thaw do not match records
No viable embryos after warming
Mislabeling of patient samples
Incorrectly or inadequately sealed carrier
Damage to carrier in transit prior to receipt at site
Loss of shipment in transit
Failure to provide warming instructions
Injury or death
Inadequate safety training of laboratory personnel
Inadequate personal protective equipment
Lack of disaster plan addressing asphyxiation prevention, frostbite
Lack of alarm system, buddy system or other safety safeguards
Other
Sabotage and deliberate destruction of records or inventory
Failure to discard samples when patient has provided adequate written consent
Failure to follow written protocols for abandoned specimens.
Failure to maintain patient contact information
Inadvertent discard of embryos deemed abandoned

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SUMMARY

Managing cryopreserved reproductive tissues in an IVF laboratory requires skilled personnel and specialized equipment. This document can be used by laboratories as guidance for developing clinic-specific tissue storage and management policies and procedures. Any tank that exhibits a loss of LN_2 that is faster than expected should be replaced as soon as possible. However, it is understood that adherence to even the most rigorous policies and procedures cannot prevent a rare catastrophic event from occurring.

Acknowledgments: This report was developed under the direction of the Practice Committee of the American Society for Reproductive Medicine in collaboration with the Society for Reproductive Biologists and Technologists (SRBT) and the Society for Assisted Reproductive Technologies (SART) as a service to its members and other practicing clinicians. Although this document reflects appropriate management of a problem encountered in the practice of reproductive medicine, it is not intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs of the individual patient, available resources, and institutional or clinical practice limitations. The Practice Committee and the Board of Directors of the American Society for Reproductive Medicine, SRBT, and SART have approved this report. This document was reviewed by ASRM members and their input was considered in the preparation of the final document. The following members of the ASRM Practice Committee participated in the development of this document: Alan Penzias, M.D.; Kristin Bendikson, M.D.; Tommaso Falcone, M.D.; Susan Gitlin, Ph.D.; Clarisa Gracia, M.D., M.S.C.E.; Karl Hansen, M.D., Ph.D.; Micah Hill, D.O.; William Hurd, M.D., M.P.H.; Sangita Jindal, Ph.D.; Suleena Kalra,

M.D., M.S.C.E.; Jennifer Mersereau, M.D.; Randall Odem, M.D.; Catherine Racowsky, Ph.D.; Robert Rebar, M.D.; Richard Reindollar, M.D.; Mitchell Rosen, M.D.; Jay Sandlow, M.D.; Peter Schlegel, M.D.; Anne Steiner, M.D., M.P.H.; Dale Stovall, M.D.; and Cigdem Tanrikut, M.D. The Practice Committee acknowledges the special contribution of Sangita Jindal, Ph.D.; Matthew "Tex" VerMilyea, Ph.D.; Arthur Chang, Ph.D.; Michael Vernon, Ph.D.; Rebecca Krisher, Ph.D.; Tom O'Leary, Ph.D.; Shane Zozula, B.S.; Mark Dow, Ph.D.; Scott E. Smith, Ph.D.; Linda Morrison, B.S.; and Marybeth Gerrity, Ph.D. in the preparation of this document. All Committee members disclosed commercial and financial relationships with manufacturers or distributors of goods or services used to treat patients. Members of the Committee who were found to have conflicts of interest based on the relationships disclosed did not participate in the discussion or development of this document.

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