INTERNATIONAL SOCIETY FOR BIOLOGICAL AND ENVIRONMENTAL REPOSITORIES

BEST PRACTICES:
Recommendations for Repositories
Fourth Edition

ADDENDUM 1:
Liquid Nitrogen-Based Cryogenic Storage of Specimens

The Liquid Nitrogen-based Cryogenic Storage of Specimens Best Practices Addendum is a collaboration between the International Society for Biological and Environmental Repositories and the Society for Cryobiology that reflects the collective experience and knowledge of industry experts.

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INTRODUCTION

The availability of high-quality biological and environmental specimens for research purposes requires the development of standardized methods for collection, handling, storage, retrieval, and distribution. The International Society for Biological and Environmental Repositories (ISBER) is the leading global forum for the development, management, and operations of repositories. One of the key objectives for ISBER is to share successful strategies, policies, and procedures on providing fit-for-purpose specimens for research. For more information about ISBER see www.isber.org.

ISBER Best Practices: Recommendations for Repositories (Best Practices) is a guidance document that reflects the collective experience of its members and has received broad input from other repository professionals. Throughout the document, effective practices are presented for the management of specimen collections and repositories and the term "Best Practice" is used in cases where a level of operation is indicated that is above the basic recommended practice or more specifically designates the most effective practice. It is understood that physical location or financial constraints can make "Best Practices" difficult or impossible to attain. Repositories facing such challenges should decide how best to incorporate these recommendations. While adherence to ISBER Best Practices is voluntary, it is important to note that some aspects of specimen management are governed by national/federal, regional, and local regulations. The reader should refer directly to their own national/federal, regional, and local regulations and requirements, as appropriate.

This addendum builds on the foundation established in previous editions of the Best Practices (published in 2005, 2008, 2012, and 2018) and is focused on the liquid nitrogen (LN_2)-based cryogenic storage of biological and environmental specimens for research and clinical use. The recommendations in this document cover topics related to facility planning and design; installation and operation of LN_2-based cryogenic storage systems and instruments; safety, training, and quality management issues; equipment relocations; disaster planning/risk management issues; and appropriate storage containers and labels for LN_2 storage.

ISBER recognizes that the definition and subsequent use of the terms "specimen" and "sample" varies when used in a clinical versus biodiversity and/or environmental setting. Definitions for each context are provided in the glossary. While "specimen" is used predominantly throughout the document, it could be substituted for "specimens and/or samples" in most instances.

ISBER has strived to include terminology and definitions that are harmonized with international standards and are universally understood. Important terms within the document are italicized when first used in a section and defined in the glossary.

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1.1 GENERAL

Specimens are commonly collected at one location and used at a later time at another location. Specimen integrity is maintained only if the critical biological properties are preserved during *storage*. The selection of a storage temperature is a function of the freezing characteristics of the water in the specimen as well as the biochemical activity of molecules present that act to degrade the specimen[1].

For many biological systems, the solution containing the specimen is complex and therefore they do not solidify at a single temperature but freeze over a range of temperatures. It is common for cells to be frozen in complex mixtures that contain cryoprotective agents[2]. The water in this type of specimen is not completely immobilized until the specimen achieves the *eutectic temperature* or partially vitrifies.

All biological specimens contain degradative molecules whose behavior is strongly influenced by temperature. Specifically, reduced temperatures result in reduced protein dynamics/activity. Optimal storage temperatures should be selected below the threshold temperature for activity of the protein. At temperatures below the *glass transition temperature* (*Tg*) of pure water (-132 °C), the extreme cold arrests biological life and slows most chemical and physical reactions that cause specimens to deteriorate.

Storing specimens at low temperatures is typically achieved using *liquid nitrogen*-based cryogenic storage. Specimens may be stored in either liquid or vapor phase of liquid nitrogen (LN_2). These LN_2 storage units differ greatly from a conventional mechanical freezer in how the specimen is cooled and factors that influence proper operation of a LN_2 storage unit. This document is intended to address a variety of considerations when designing, operating, and maintaining a facility with LN_2-based cryogenic systems. Additional guidance and information can be found in the *ISBER Best Practices: Recommendations for Repositories 4th edition* and in a review article by Schiewe, *et al.*[3].

1.2 FACILITY PLANNING AND DESIGN

1.2.1 STORAGE EQUIPMENT TYPES

The use of LN_2 for long-term specimen *preservation* is optimal for the storage of some types of biological material. Cryogenic storage using LN_2 is an effective long-term storage platform because on-site LN_2 supplies reduce reliance on mechanical freezers that use electrical power, especially in areas where power supply is unreliable.

LN_2-based storage units can be divided into two main groups, smaller aluminum *Dewars* and larger *storage units* (*i.e.*, vessels, freezers) housed in stainless steel, powder-coated steel, or plastic. Both are double-walled, vacuum-insulated storage units designed to efficiently hold LN_2 and accommodate various specimen types. Dewars and LN_2 storage units come in different sizes, specimen capacities, and with varying degrees of instrumentation and automation.

1.2.1.1 Dewars

Aluminum Dewars are small, efficient, and transportable containers that often fit conveniently in labs or under counters and are readily accessible. If properly vacuumed, Dewars provide a stable storage temperature and low LN_2 usage. Originally designed for LN_2 submersion or liquid storage, some Dewars can also be used for vapor storage by only partially filling the storage unit with LN_2. Although a few auto-fill models exist, the vast majority require routine manual filling of LN_2 to maintain temperature. Add-on temperature and LN_2 level monitoring accessories are available, but Dewars typically lack the full monitoring and LN_2 level control options available with larger storage units thus requiring a higher level of manual management and verification.

1.2.1.2 Liquid Storage Units

These are medium to large storage units designed for long-term storage of specimens. Depending on their size, they may fit in a lab or require a dedicated room, facility, or *repository*. Although completely manual units exist, the vast majority have auto-fill capabilities with instruments for temperature and LN_2 level and usage monitoring. These instruments and systems can provide convenience and consistency but still require manual verifications at regular, pre-determined intervals to ensure specimen integrity.

Technological advances have led to the development of new systems that allow specimens to be handled either fully or semi-automatically while stored in the vapor phase of LN_2. Such systems provide areas for handling and *retrieval* that are cooled to ultra-low temperatures (below -100 °C) preventing cooling/re-warming cycles of the specimens. Liquid storage units can be divided into two main groups: "open-top" and "high-efficiency" storage units.

1.2.1.2.1 Open-top Units

Open-top storage units have a large lid that provides access to an open storage space. Originally designed for LN_2 submersion or liquid storage, some models can also be used for vapor storage by only partially filling the storage unit with LN_2 and placing specimens on a tray above the LN_2. Open-top storage units are often less expensive but have a higher operating cost and LN_2 usage due to the large lid opening and additional heat input. The larger lid opening can facilitate easier specimen access especially in a high-throughput workflow. When using open-top storage units in vapor storage, consideration should be given to the storage temperature gradient. The top temperature can vary significantly depending on the LN_2 level, frequency and duration of access, and model type. In addition, the full-to-empty or full-to-critical time for these systems can be as little as 48 hours.

1.2.1.2.2 High-Efficiency Units

These storage units have a smaller, offset lid that provides access to the storage space while maintaining significantly more vacuum-insulated surface compared to an open-top unit. This provides temperature performance independent of LN_2 level and lid openings with very low LN_2 usage. These models are equipped with an interior turn tray or "Lazy Susan" to allow access to specimens. The turn tray is above a reservoir of LN_2 and keeps specimens in the dry storage space. Vaporization of the LN_2 reservoir within the insulated storage unit provides cryogenic storage temperatures and routinely maintains a -190 °C vapor temperature.

Storage in LN_2 vapor phase (≤ -150 °C) is preferred to submersion in liquid phase (-196 °C) because it provides sufficiently low temperatures to maintain

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specimens below the *Tg* while avoiding contamination issues and *safety* hazards inherent in liquid phase storage.

1.2.1.3 Dry Storage Units

These storage units do not utilize liquid in the specimen storage area (*i.e.*, "dry" units), reducing the risk of specimen contamination and the safety risks to personnel associated with the handling of liquid nitrogen while still providing a consistent temperature profile associated with LN_2-based storage.

Best Practice: Appropriate liquid level and temperature monitoring equipment as well as manual monitoring should be utilized for all storage of biological material.

Best Practice: Divide invaluable or irreplaceable specimens between storage units to reduce risk of loss.

1.2.2 FACILITY CONSIDERATIONS

An efficient repository has many particular location and design elements to ensure the safekeeping of the material stored, support the equipment employed, and provide a safe and effective working environment for the repository staff. In planning the design of a repository, it is necessary to determine the types of material being stored, the required storage and handling conditions, the projected retention periods, projected growth of the stored specimen numbers, and the projected use of the materials. The design should include sufficient space to accommodate the material planned for initial, future, and backup storage and also provide for the safe movement of people, equipment, and specimens, as needed, or as required by law and/or other regulatory agencies. This may include an environmental assessment to provide evidence that the facility during its routine operations won't negatively affect the environment and/or people around it, particularly if the LN_2 supply tank is located outside the main repository building. See *ISBER Best Practices: Recommendations for Repositories 4th edition, Section B: Facilities* for additional information.

Best Practice: Planning for a repository within a facility should take into account the environmental conditions experienced by the region where it is located (*e.g.*, fire, flooding, high winds, earthquake, tsunami) and availability of resources (*e.g.*, stable electricity, liquid nitrogen) and a disaster recovery plan should be established for the repository.

1.2.2.1 Space

Adequate space should be allotted with sufficient clearance per manufacturer recommendations for operation, routine verifications, maintenance, and cleaning of both cryogenic equipment itself and of auxiliary equipment (*e.g.*, air conditioners, monitoring and safety equipment). This includes the location area and height clearance of both the equipment and any specimen racks being placed or removed. Space design should allow for the physical removal of any individual storage unit within the space without the requirement to move other storage units in the area. Consideration should be given to the width and height of all doorways, hallways, elevators, and any pass-through between the loading dock and storage location. Space outside the repository should be sufficient for delivery of LN_2 from the local manufacturer by a specialized truck (*e.g.*, sufficient road structure, adequate parking space).

1.2.2.2 Flooring and Structural Support

Flooring surfaces used in repositories should be appropriate for the equipment and refrigerants used in daily repository activities. Flooring should be easy to clean and facilitate the movement of equipment when circumstances warrant. Special consideration should be given to the flooring where LN_2 is used (*i.e.*, vinyl tile should not be used as it will crack and cause a hazard if liquid nitrogen is spilled directly onto it). Consideration of the weight and vibration of large cryogenic units should also be considered as over time the units will wear away at floor surfaces due to the point-pressure applied by the casters. Repositories should consider providing anti-fatigue mats for staff in areas where personnel stand for prolonged periods of time. Due to the high weight of storage and other equipment (*e.g.*, freezers, LN_2 storage units, heavy cabinets), consideration of the combined weight of the equipment must be taken into account when locating the repository within a building or when designing a new facility.

Seismic bracing may be required depending on the geographical area. Local guidelines and regulations for seismic bracing of LN_2 storage units, supply tanks, and/or piping systems should be followed. Pad location and build for bulk tanks are required to conform to local seismic build regulations for the category of the location.

1.2.2.3 Air Flow, Circulation, Temperature, and Humidity

Sufficient air circulation should be provided to prevent excess moisture and condensation. Excess humidity can lead to fungal growth if left unchecked, which may affect specimen integrity and cause health problems for staff. Air circulation should be managed to minimize air passing over the opening of a storage unit which accelerates nitrogen boil off. In addition, the constant refreshment of moist air can lead to significant icing issues on the sides of the storage units. Adequate ventilation and monitoring are also critical in repositories where LN_2 and *dry ice* are used to ensure that sufficient oxygen levels are maintained (see *ISBER Best Practices: Recommendations for Repositories 4th edition, Section C2.3.1: Oxygen Sensors*). Specific requirements for regular and emergency ventilation for LN_2 storage areas exist in some countries.

In most repositories it is critical to maintain ambient temperature within defined limits. Sufficient heating and air conditioning capacity should be provided to maintain the repository temperature and control humidity to maximize equipment life.

Best Practice: Ensure any storage unit cooled with LN_2 has unobstructed venting that is regularly checked for frost or ice buildup.

Best Practice: Appropriate monitoring devices (*e.g.*, oxygen monitors) with both auditory and visual alarms should be combined with a dedicated exhaust system and installed within areas where low oxygen level might develop or harmful gases might accumulate. This system provides a sufficient amount of recirculating air to replace the air volume of a room according to the local regulations. The extracted gases are vented to the outside of the building, according to regulations, and never to the interior areas of the building.

Best Practice: Repositories located in areas where humidity is high (*e.g.*, coast) should employ a de-humidification system to ensure optimal operation of the equipment.

1.2.2.4 Electrical Power

Although LN_2-based storage units do not rely on electrical power for active cooling, it is required for monitoring, alarms, and replenishment of LN_2 for auto-fill systems. Backup power or battery backup is recommended and should be able to provide sufficient monitoring and alarm capabilities to last through weekends or any duration where the facility is unmanned. Battery backup should be capable of providing one complete filling cycle.

1.2.2.5 Network Reception

Consideration should be given to the cellular and/or wireless internet reception in the repository. LN_2 storage units, supporting systems, and instruments may require cellular or internet connections for alarm notifications. Using wired connections or local repeaters can assist with a reliable network connection in buildings or rooms with insufficient reception.

1.2.3 LN_2 SUPPLY

LN_2 is a consumed refrigerant and must be regularly replenished to maintain the cold storage capacity. The LN_2 supply system should be scaled to provide sufficient LN_2 for a minimum 21-day supply at normal usage and replenishment intervals should be maintained with the assumption that a re-supply is readily available.

Best Practice: Bulk tanks, delivered cylinders, and LN_2 supply volume and pressure should be verified on a regular basis. All LN_2 connections should be routinely checked to ensure they are tight and leak free.

1.2.3.1 Bulk Supply Systems

For bulk tank supply systems, vacuum-jacketed withdrawal, piping, and valves are recommended to increase efficiency by lowering overall system LN_2 consumption and providing fast and consistent LN_2 to the storage units. They also increase safety by avoiding cold surfaces, condensation, and wet, slippery floors.

A *telemetry system* may be installed to allow suppliers to monitor liquid levels in real time to ensure stocks do not drop below agreed upon threshold levels. Bulk storage and piping systems require relief valves to prevent rupturing of the pipe and bulk tanks in the event of over-pressure. A line pressure gauge should be installed within the facility to use as a reference for pressure build events and for troubleshooting longer than normal fill times from

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the bulk system. If relief valves trip unexpectedly, a person near a valve can be sprayed with either the cold gas or the liquid. In the event of a blockage or excessive pressure, several relief valves may vent nearly simultaneously, causing a "whiteout" condition in a matter of a few seconds. Visibility can drop to near zero and oxygen levels in the area may become less than that necessary to sustain life. Under these circumstances, personnel should evacuate immediately.

Oxygen monitoring should be installed by a professional company familiar with LN_2 (*e.g.*, a bulk gas supply company) in any areas of the facility where LN_2 is utilized (see *ISBER Best Practices: Recommendations for Repositories 4th edition, Section C2.3.1: Oxygen Sensors*). A vacuum-insulated and pneumatically actuated shut-off valve should be located outside at the bulk tank and tied into the oxygen detection system for immediate termination in the event of a dangerous oxygen level inside the building (see Section 1.2.4.1.1 Oxygen Monitoring).

For bulk supply tanks with piping systems, consideration should be given to staggering the pressure rating of relief valves on the LN_2 supply tank, piping, and storage units so that the primary relief should be set in such a way as to be outside the building with the lowest value at the bulk tank. Measures should be in place to prevent over-pressurization of the bulk tank during filling (*e.g.*, bottom tri-cock can be locked off). Internal reliefs can be piped away to external vents and be directed away from personnel and throughways to avoid unintentional liquid or gas sprays during over-pressure events. An external pneumatic valve connected to the oxygen alarm system and panic buttons should be fitted at the outlet of the bulk tank.

1.2.3.2 Portable Supply Tanks

Portable supply tanks require regular delivery depending on the LN_2 storage unit. The LN_2 volume and pressure of these portable supply tanks needs to be monitored often to ensure adequate supply. These portable tanks also require the LN_2 storage unit transfer hose to be routinely disconnected and reconnected. Special care should be given during this process to avoid introducing moisture, dust, and debris into the supply system. Supply hoses should never be changed when cold (frosted).

The proper pressure rated relief valves should always be confirmed for the specific application. Manual checks of gauges and indicators on supply tanks are required as they are often inaccurate and unreliable.

For portable supply cylinders, vacuum-jacketed flex hoses are recommended. These will increase efficiency by lowering overall system LN_2 consumption and providing faster and consistent LN_2 to the storage units. They also increase safety by avoiding cold surfaces; condensation; and wet, slippery floors. Noting the exchange frequency of the supply cylinder is helpful as an indication of a vacuum issue or leak in the plumbing system or perhaps that a setting has been changed on the LN_2 storage unit.

1.2.4 SUPPORTIVE SYSTEMS

1.2.4.1 Personnel Safety Monitoring

1.2.4.1.1 Oxygen Monitoring

When LN_2 evaporates into the air, oxygen molecules are displaced which can cause potential life-threatening asphyxiation if proper safeguards are not in place to alert and notify staff of the issue ensued by immediate evacuation.

Accompanying all oxygen monitors should be proper signage to clearly explain what the alert is for and what the potential risk is. Oxygen monitors should be installed and used in all areas exposed to LN_2 in the following manner:

- Installed inside the immediate areas exposed to LN_2.
- Sensor placement should follow manufacturer recommendations based on the size of the area to be monitored and should not be placed in the path of the water vapor plume that forms during the filling of a LN_2 storage unit as it will cause the oxygen sensor to alarm.
- Outside the area to alert others not to enter in case of an oxygen deficiency.
- Outside of the building to alert staff/first responders.

Oxygen monitors with both an audio (beeping/horn sounds) and a visual (strobes/flashes)

function should be installed to indicate when the oxygen level is below the acceptable range. In most cases, an oxygen monitoring system will allow for multiple notification settings at various oxygen levels to indicate a slightly out-of-range condition that may not cause immediate danger vs. a significant out-of-range condition that is likely to cause immediate danger.

If the oxygen monitor has only one setting for an alarm, the alarm function setting should occur at 19.5% oxygen. At this level, there is an out-of-range condition (given that standard atmospheric air contains ~21% oxygen) and action should be taken to remove staff from the immediate area to a safe environment until the oxygen level has returned above 19.5%. If the oxygen monitor has two or more settings, the first should be set to 19.5% (as indicated above) and the second setting should be set at ~17.5%. In this scenario, there is a significant oxygen level deficiency that requires immediate evacuation of staff to a safe environment until the oxygen level has returned above 19.5%. Generally, an automated emergency ventilation system is triggered by alarm associated with low oxygen level.

When responding to an alert, if oxygen levels are between 19.5% and 17.6%, the LN_2 supply should be turned off and all filling into the area stopped. Personnel should exit the immediate area into a predetermined safe environment (outside of a building is recommended). Personnel should only return to the oxygen-deficient area once the oxygen level has recovered above 19.5% and management has given the all-clear. If oxygen is 17.5% or lower, personnel should immediately evacuate the area into a predetermined safe environment (*i.e.*, outside the building) and only return to the oxygen deficient area once the oxygen level has recovered above 19.5% and management has given the all-clear.

An oxygen sensor placed in a repository at high altitude should have a normal reading of 20.9%; however, the decreased total air pressure at higher altitudes means that each breath of air delivers fewer oxygen molecules to the body. This can be mathematically converted into an "effective oxygen percentage". For this reason, repositories located at high altitudes may consider tightening the thresholds for their oxygen sensor alarms.

Both fixed and mobile/personal monitors may be appropriate depending on the size of the facility. Even when installed units indicate an alarm condition, it may be useful to employ a personal monitor to enter the room carefully to validate the alarm condition if the area is not visible from outside the room. It may be more appropriate to use mobile oxygen monitors in a secure area where LN_2 freezers operate because the sensors in installed units will degrade over time and sound false alarms.

1.2.4.1.2 Curved Mirrors and Cameras

Methods of seeing into the repository are helpful for visual inspection of LN_2 storage units, as well as identifying staff during a time of emergency. Correctly positioned, curved mirrors and cameras assist in observing if anyone has collapsed in the facility and is on the floor (due to low oxygen or other issues). Additionally, internal doors may have transparent glass areas to allow see-through.

1.2.4.2 Specimen Integrity Monitoring

A thorough monitoring system should be implemented into the facility design to alert staff of urgent issues needing prompt response to prevent specimen loss. This system should provide monitoring for specimen storage conditions (*i.e.*, temperature) and storage equipment functionality (*i.e.*, LN_2 consumption) to mitigate the risk of equipment failure. The monitoring system should include multiple redundant sources of both human and electronic components.

Best Practice: Active monitoring as well as regular manual monitoring (*i.e.*, daily, weekly) should be performed to reduce risk of catastrophic loss. Additionally, such specimens should be divided between LN_2 storage units to reduce risk of loss.

1.2.4.2.1 Frequency of Monitoring

Temperature monitoring should be performed and recorded continuously. Frequency of all other monitoring elements should be determined based on the influences of the metric.

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1.2.4.2.2 Monitoring Methods

There are two primary methods of monitoring: manual monitoring and electronic monitoring. Both manual monitoring and electronic monitoring have a multitude of benefits and, when combined together, offer a complete monitoring system with built-in redundancy.

- Manual monitoring should be performed at a minimum to verify the temperature reading/display of a reference/certified/traceable thermometer, LN_2 level, LN_2 consumption, and visual observations (*i.e.*, condensation or ice on the outside of the LN_2 storage unit). The benefits of manual monitoring include, but are not limited to:
  - » Provides visual observation of the condition of the LN_2 storage unit and specimens that electronic monitoring cannot replace.
  - » Not reliant on electrical power or additional sources of technology that may fail if not properly validated and maintained or in the event of a natural disaster.
- Electronic monitoring should be used to monitor at a minimum for temperature, LN_2 level, and LN_2 consumption. The benefits of electronic monitoring include, but are not limited to:
  - » Acts as an early detection/notification system to alert staff 24 hours a day, 7 days a week, and 365 days a year.
  - » Can be achieved via a local, storage unit-specific, monitoring system that is built into the LN_2 storage unit.
  - » Can be achieved via a 3rd party call-out system.
  - » Allows for prompt response by staff for issues that potentially wouldn't be discovered by manual monitoring for several hours.
  - » Continuously monitors and records values to ensure specimen and equipment integrity.
  - » Can generate historical reports and trending.

1.2.4.2.3 Failure Detection

Detection methods for LN_2 storage unit equipment failure leading to potential specimen loss or compromise generally include abnormal LN_2 consumption, abnormal visual observations, and abnormal temperatures.

- LN_2 consumption is often used as a leading indicator (slow equipment failure over time) as well as an indicator in urgent situations.
- Visual observation should be monitored, recorded, and reviewed at regular intervals to indicate potential equipment failure. Visual observation should include the following:
  - » Checking for obvious damage on the inside and outside of the LN_2 storage unit for such things as dents, warping, or other abnormal findings that are inconsistent with the way the storage unit normally looks/functions.
  - » Checking for condensation or ice on the outside of the LN_2 storage unit (when it hasn't been filled recently) which may indicate a vacuum leak and/or imminent failure.
- Temperature monitoring should be used to indicate when LN_2 level is low and/or equipment is not functioning properly. A temperature alert is sometimes the last indication of equipment failure. Typically, a LN_2 supply alarm will activate and be followed by a low level alarm before the temperature alarm is activated. Response time to a temperature alert should be within an hour or less to prevent specimen loss.

If a LN_2 storage unit is suspected of being in a state of failure or is failing, personnel should ensure the LN_2 level is maintained and remove material immediately. Allowing the LN_2 level to drop during a failure may result in the implosion of the interior wall which will prevent the removal of the material being stored.

1.2.4.2.4 LN_2 Level Monitoring

LN_2 level should be monitored and recorded manually to the nearest quarter inch (0.25 inches or 6 mm). When being performed manually, a plastic or metal yard stick is typically used. Other devices of various materials may be used but should be evaluated on how well they hold up to exposure to cryogenic temperatures. A hollow tube should never be used as LN_2 will rapidly shoot out of the top as it is inserted into the cold LN_2. When there is an electronic component that measures LN_2 level, the electronic level should be monitored and recorded. The manually measured LN_2 level should be compared within a predefined acceptable range (recommended 1" as the acceptable *deviation*). If out of the acceptable range, the electronic LN_2 level should be calibrated to match the physically measured level. If a manual-filled LN_2 storage unit is being used (as opposed to an auto-fill LN_2 storage unit), the LN_2 level should be monitored and recorded before filling. An additional measurement of post-fill levels allows for the calculation of LN_2 consumption and determination of storage unit evaporation rates.

1.2.4.2.5 LN_2 Consumption Monitoring

LN_2 consumption should be measured and recorded manually (for manual fill LN_2 storage units) and electronically (for auto-fill LN_2 storage units). For manual fill LN_2 storage units, wireless remote, weight-based monitoring systems can provide continuous measurements that can be precisely correlated to LN_2 levels and used to determine evaporation rates. An alternative method is to take the current LN_2 level and subtract that from the last recorded LN_2 level (after filling the storage unit). If performed daily, this will provide consumption of LN_2 for a specific storage unit that can be averaged out with standard deviations. On auto-fill LN_2 storage units, the electronic monitoring system is used to provide this information. Since auto-fill LN_2 storage units generally fill on an "as needed" basis, it is difficult to determine the LN_2 usage manually. The digital/electronic component is the best source for tracking and recording LN_2 consumption. LN_2 consumption monitoring should include a clear acceptable range of consumption with a calculated standard deviation, as identified by the manufacturer's guidelines and in accordance with the facilities average usage of the LN_2 storage unit.

Best Practice: LN_2 consumption should be monitored, recorded, and reviewed at regular, defined intervals (*i.e.*, daily, weekly) to assess potential equipment failure. An increased LN_2 consumption rate beyond manufacturer guidelines and/or the standard consumption rate at the user's facility is a critical sign that equipment should be retired or that failure is imminent and action should be taken immediately.

1.2.4.2.6 Temperature Monitoring

LN_2 storage units should have continuous temperature monitoring by a reference/certified/traceable thermometer. Even when specimens are completely submerged in LN_2, temperature monitoring can provide reassurance that specimens have maintained a proper temperature if/when LN_2 level falls out of range. Acceptable ranges should be clearly identified for both high and low values and the temperature limit should be determined based off the material being stored.

A redundant (independent) reference/certified/traceable thermometer should be placed at the highest level of the specimens being stored within the LN_2 storage unit (or higher to give earlier warnings/notifications). If multiple temperature sensors/probes are set-up for redundancy at the same height within a given LN_2 storage unit, a clearly defined acceptable deviation range should be identified. A comparison of the two within the acceptable range should be monitored and recorded to ensure they are functioning properly.

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1.3 INSTALLATION AND MAINTENANCE

1.3.1 INSTALLATION

The proper installation and operation of LN_2-based cryogenic storage systems and instruments should be verified prior to use per manufacturer recommendations and as determined as fit-for-purpose with the repository's practices.

1.3.1.1 Calibration

A system for the *calibration* of all instruments should be in place. Any device that provides analog or digital measurements is considered an instrument and requires calibration. Instruments should be factory calibrated with documentation provided or may require verification and/or calibration at the time of installation. Verification and calibration should be done annually or according to manufacturer recommendations. For LN_2 storage units, these can include temperature and LN_2 level sensors as well as any secondary systems and oxygen monitors.

Best Practice: Calibration records should include the appropriate standard readings taken both before and after calibration.

Best Practice: A log of calibration records should be kept that includes the date of the calibration, the name of the individual performing the calibration, the name/serial number of the device used against which the instrument is calibrated, and a reference to the standard operating procedure (SOP) used to perform the calibration.

Best Practice: Instruments used for calibrations should be verified against an approved, recognized calibrator source.

1.3.1.2 Verification

Proper installation and operation of cryogenic storage systems and instruments should be verified per manufacturer recommendations. For LN_2-based cryogenic storage systems, this should include:

- Documentation – user/service manuals, calibration certificates, maintenance logs, and any factory acceptance documentation which should be stored in a known location available to personnel.
- Contact information for key personnel – should be logged and stored in a known location. Should include personnel from the user group, facilities, LN_2 supplier, equipment manufacturer, and service provider.
- Training and SOPs – a training program should be in place for all personnel on safety, routine verifications, emergency response plans, and all other SOPs.

1.3.1.2.1 Temperature Mapping

LN_2 storage unit temperature mapping is typically performed by the manufacturer as part of design qualification. Verification should be performed by the user using a secondary sensor to verify the temperature at a specified location within the storage unit, typically at the highest level at which specimens will be stored. Some customers may require temperature mapping to be performed on-site by the service provider before a unit is put into service, which may take 2-4 weeks per unit depending on customer requirements.

1.3.1.3 Installation and Setup

Proper installation and setup should be verified and documented using manufacturer recommendations for electrical supply, LN_2 supply, and facility requirements. Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) or IQ/OQ/PQ are independent and documented *procedures* used together to verify and test that a storage unit is installed correctly, meets its design requirements and specifications, and operates the way in which it was designed under load. Installation Qualification is the oversight and verification of every physical aspect of the equipment (materials, dimensions, pressure ratings, etc.) and components (operational parameters, accuracy, voltage, etc.). Operational Qualification is the testing of each individual component/feature/physical specification of the equipment. Performance Qualification, much like Operational Qualification, tests the operational requirements of the equipment but is performed under conditions which the equipment would be subject to during use (i.e., "real-world" conditions).

Some countries require certification of all equipment under pressure (*e.g.*, LN_2 supply equipment) by a special governmental body or agency. Refer to national/federal, regional, and local regulations and requirements, as appropriate.

1.3.1.3.1 Electrical Power

Although LN_2-based cryogenic storage units do not rely on electrical power for active cooling, it is required for monitoring, alarms, and replenishment of LN_2 for auto-fill systems. Backup power or battery backup is recommended and should be able to provide sufficient monitoring and alarm capabilities to last through weekends or any duration when the facility is unmanned. Batteries used in battery backup systems should be changed per the manufacturer recommendation. The battery backup system should have enough power to complete one fill cycle.

1.3.1.3.2 LN_2 Supply

The LN_2 supply system should be scaled to provide sufficient LN_2 for a minimum 21-day supply at normal usage and replenishment intervals. In some regions, the off-site provider is located at a distance too far from the repository to ensure re-supply within 21 days. In this case, installation of a backup LN_2 supply system is recommended, such as a portable LN_2-producing plant with a smaller capacity to allow maintenance of normal repository functions for a minimum of 10 days. Bulk tanks or delivered cylinders and LN_2 supply volume and pressure should be verified on a regular basis. All LN_2 connections should be routinely checked to ensure they are tight and leak free.

For bulk tank supply systems, vacuum-jacketed withdrawal, piping, and valves are recommended. For supply cylinders, vacuum-jacketed flex hoses are recommended as they increase efficiency and provide a fast and consistent LN_2 supply to the storage units. See *ISBER Best Practices: Recommendations for Repositories 4th edition Section C2.2: Liquid Nitrogen Supply* for additional information.

1.3.1.3.3 Pressure Relief Valves

Pressure relief valves are necessary when dealing with any type of cryogen. Nitrogen has a liquid to gas expansion ratio of 1:696. Relief valves must be present on any LN_2 storage unit, tank, or section of piping that potentially can trap LN_2 to avoid explosion hazards. Pressure relief valves should be piped to the outside and verified for correct pressure rating and operation. Relief valves should be replaced on a preventative maintenance schedule and as needed.

1.3.1.3.4 Secondary Monitoring

If a secondary system for monitoring temperature and/or LN_2 level will be used, it should be installed, calibrated, and verified prior to introducing specimens.

1.3.1.3.5 Initial Fill

Following verification of proper installation and setup, the LN_2 storage unit should be filled with LN_2 per manufacturer recommendations and allowed to stabilize for 24-48 hours prior to introducing specimens. The inventory rack system should also be installed during this time.

Best Practice: IQ, OQ, and PQ should be performed on the unit prior to specimen introduction.

1.3.1.4 Operation

1.3.1.4.1 Temperature

A stable storage temperature measured at the identified warmest point should be verified using an independent, calibrated sensor prior to introducing specimens. Temperature should be monitored continuously and recorded at frequent, regular intervals.

1.3.1.4.2 LN_2 Level

LN_2 level should be monitored and recorded. When there is an electronic component that measures LN_2 level within the storage unit, readings should be manually confirmed and compared to primary and secondary monitor readings for accuracy. For auto-fill systems, proper functionality (including any instruments, decision-making, and valve operation) should be verified.

1.3.1.4.3 LN_2 Usage

It may take several days of normal operation for some systems to establish a baseline LN_2 usage. This value should be recorded and referenced to

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verify LN_2 storage unit performance. This baseline value can vary significantly between LN_2 storage units depending on the size and type. An increase in LN_2 usage can be the first indication of a LN_2 storage unit problem or potential failure.

1.3.1.4.4 Backup Power

Backup power or battery backup operation should be verified, if present. If only limited power backup is available, priorities for power should be established in advance.

1.3.1.4.5 Alarms and Alerts

Alarms and alerts should be verified and tested. This includes local audio visual alarms as well as any secondary or remote notification systems.

Best Practice: Manufacturer's recommendations and guidelines for installation and operation should be followed.

Best Practice: The entire system should be re-validated when any changes are made to the unit, software, and/or automation equipment.

1.3.2 ROUTINE MAINTENANCE

A system for preventative maintenance and repair of LN_2-based storage equipment and supporting systems should be in place. System maintenance should be performed at regular, established intervals per manufacturer's recommendation and as determined as fit-for-purpose aligned with the repository's practices.

It is important to communicate with manufacturers and service providers regarding the importance of the specimens being stored as well as the surrounding workflows so they can help develop appropriate verification and maintenance schedules. Critical specimens may require a more aggressive program with emphasis on the preventative maintenance. LN_2 storage units that are accessed more frequently may also require an accelerated maintenance schedule.

Best Practice: Preventative maintenance should be performed by factory-trained and/or certified technicians at regular, established intervals per manufacturer recommendations and as determined as fit-for-purpose aligned with the repository's practices.

1.3.2.1 LN_2 Storage Units

1.3.2.1.1 Manual Verifications

Depending on the storage system, daily or weekly manual verification of storage temperature, LN_2 level, and LN_2 usage is recommended. Records of manual verifications should be kept and referenced to observe any developing trends or changes in system performance and made available for *audits*.

1.3.2.1.2 Condensation, Frost, and Ice Buildup

Maintaining a dry environment in the repository reduces condensation and frost or ice buildup. Ice buildup can affect performance and usability of storage units. Routinely wiping surfaces dry and removing ice buildup on the lid will improve efficiencies and increase equipment life.

Excessive moisture can lead to ice buildup inside the LN_2 storage unit that can interfere with auto-fill and LN_2 level measurement and accelerate the need for units to be taken out of service, warmed, dried, and cleaned. LN_2 storage units should be routinely inspected for excessive condensation and frost along vacuum surfaces that may indicate a problem with the insulation.

1.3.2.1.3 LN_2 Connections

All LN_2 connections should be routinely inspected to ensure they are tight and leak free. Ice balls are indicators of leaks and should be eliminated through tightening connections where found.

1.3.2.1.4 Backup Power

Backup power or battery backup should be routinely verified for proper functionality. Batteries have a limited shelf-life and should be replaced per manufacturer's recommendations.

1.3.2.1.5 Alarms and Alerts

Alarms, secondary monitoring, and remote alert systems should be routinely verified. Steps for performing system verification should be outlined in an SOP and results should be documented, maintained, and available for audit.

Best Practice: Alarms should be tested on a regular basis (*e.g.*, weekly or monthly) to ensure proper functioning and call-out to notification devices used by staff that are "on call".

1.3.2.1.6 Steps, Handles, and Lids

User interface components such as steps, handles, and lids should be routinely inspected for wear and tear and verified for proper functionality. Lid gaskets should be inspected for proper seal and venting and replaced if damaged or deteriorated. Poor lid gaskets can lead to inefficiencies and excessive ice build-up.

1.3.2.1.7 Plumbing Components

For auto-fill systems, plumbing components should be routinely verified and replaced per manufacturer's recommendations. These can include pressure relief valves, fill valves, and any instruments.

1.3.2.1.8 Thaw, Clean, and Dry

LN_2 storage units may require regular thawing, cleaning, and drying to maximize efficiency and lifespan. Manufacturer's recommendations should be followed for schedule as well as approved cleaning agents and protocols to avoid equipment damage. Special care must be taken when potentially infectious or hazardous specimens are being stored. Precautions must be taken during the transfer of specimens between units to protect them from adverse transient warming which could affect specimen *quality.*

1.3.2.2 Electronics

Many LN_2 storage units include built-in or add-on electronics. These electronic and electromechanical components and sensors measure temperature(s) and LN_2 level and also control LN_2 for auto-fill systems. These components have a significantly shorter design life than the LN_2 storage unit itself. Manufacturer and service provider recommendations should be followed for calibration and replacement of these components.

Best Practice: Battery backup should be should be replaced on a scheduled, routine basis.

Best Practice: Cryogenic unit electronic components and sensors should be routinely verified for accuracy and calibrated and replaced according to manufacturer and service provider recommendations.

1.3.2.3 Supply Systems

1.3.2.3.1 Manual Verifications

LN_2 supply volume and pressure gauges should be manually checked at regular, designated intervals (*i.e.*, daily, weekly) to ensure adequate supply. Records of manual verifications should be maintained and made available for audits.

1.3.2.3.2 Condensation, Frost, and Ice Buildup

LN_2 supply tanks, piping, valves and connections should be routinely inspected for excessive condensation, frost, and ice buildup. This can be an indication of an inefficiency, leak, or potential problem with the vacuum insulation.

1.3.2.3.3 Pressure Relief Valves

Pressure relief valves are necessary when dealing with any type of cryogen like LN_2. Relief valves must be present on any LN_2 storage unit, tank, or section of piping that potentially can trap LN_2 to avoid explosion hazards.

Pressure relief valves should all be vented to the outside and should be verified for correct pressure rating and operation and should be replaced on a preventative maintenance schedule and as needed.

For bulk supply tanks with piping systems, consideration should be given to staggering the pressure rating of relief valves on the LN_2 supply tank, piping, and storage units so that the primary relief is outside, piped away or in an area least likely to affect personnel.

1.3.2.3.4 Valves, Piping, Hoses, and Connections

Supply tank valves, piping, hoses, and connections should be routinely verified for proper operation and replaced per manufacturer recommendations.

1.3.2.3.5 Telemetry

Integrated telemetry systems are available for supply tanks that provide remote access to LN_2 levels, tank pressure, and line pressure. These can facilitate remote monitoring, maintenance, and fill scheduling and should be routinely verified per manufacturer recommendations.

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1.3.2.3.6 Vacuum Integrity Check

Any deterioration in vacuum insulation should be apparent by cold spots, frost, or condensation. Vacuum levels should be checked on bulk tanks and vacuum-insulated piping per manufacturer recommendations.

1.3.2.3.7 Tank Burst Disc

Supply tanks are equipped with a burst disc, also known as a rupture disc. It is a non-reseal-ing pressure relief diaphragm that protects a pressure vessel from a hazardous over pressurization. Burst discs should be routinely inspected and replaced per manufacturer recommendations.

1.4 SAFETY

1.4.1 GENERAL PRECAUTIONS

The extremely low temperature of LN_2 can cause severe frostbite and/or eye damage upon contact. Items in contact with LN_2 become extremely cold. Touching these items can result in torn flesh. Substances become brittle upon contact with LN_2 and may shatter when cold, such items are common glass and large solid plastics, which can send material flying, possibly causing an injury.

The liquid to gas expansion ratio of LN_2 is 1:696 when brought to a gaseous phase at room temperature[4] which can cause an explosion of a sealed vessel. The release of nitrogen can also displace oxygen in a room and cause asphyxiation. See *ISBER Best Practices: Recommendations for Repositories 4th edition, Section F: Safety,* for additional information.

1.4.2 SAFETY EQUIPMENT

1.4.2.1 Oxygen Sensors

Because nitrogen displaces oxygen, care should be taken when LN_2 storage units are employed. The risk is inversely correlated with the size of the room. Oxygen level sensors should always be employed when LN_2 storage units are used in a repository. Normal levels of oxygen in ambient air should be ~21%. Most installed oxygen sensor units have batteries or sensor cells that should be replaced and re-calibrated as directed by the manufacturer. Consult the manufacturer for recommended requirements to determine the number of sensors needed based on the size of the room, to confirm the placement in the room, and height of wall mounting. Acoustic and visible alarms may be installed in and out of the room and dedicated exhaust ventilation may be used in coordination with the sensors. Rooms with LN_2 storage units should have viewing windows to determine whether it is occupied during alarms.

Both fixed and mobile/personal monitors may be appropriate depending on the size of the facility. Even when installed units indicate an alarm condition, it may be useful to employ the use of a personal monitor when entering the room to validate the alarm condition if the area is not visible from outside the room. It may be more appropriate to use mobile oxygen monitors in a secure area where LN_2 storage units operate because the sensors in installed units will degrade over time and sound false alarms.

Best Practice: All monitoring systems for measuring oxygen-deficient atmospheres should be installed and evaluated per the manufacturer instructions. Staff should carry personal oxygen monitors when safety conditions warrant.

Best Practice: Duplicate systems (*e.g.*, wall system, automatic emergency fans, automatic door opening, personnel monitoring system) should be employed to ensure the highest level of personnel protection.

1.4.2.2 PPE

Those working with or around LN_2 should use appropriate protective wear and document their appropriate training. Personal Protective Equipment (PPE) required when handling LN_2:

- Safety goggles (unvented) are required at all times.
- A face shield is required when working with large volumes of LN_2 (*i.e.*, when pouring or filling).
- Cryogenic gloves which are designed to be used in the vapor phase only and should not be immersed into liquid nitrogen under any circumstances. They should be loose-fitting enough so they can be quickly removed if liquid should pour into them. Latex gloves should also be used when personnel share insulated gloves.
- A lab coat with long sleeves is required to minimize skin contact.
- Pants should be worn on the outside of shoes or boots to prevent shoes from filling in the event of spillage.
- An apron should be worn when handling large quantities of LN_2.
- Shoes should cover the entire foot and be sturdy and non-absorbent.

When not in use, all PPE should be stored in an appropriate location to ensure they do not become damaged or contaminated and are easily located by the repository staff.

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1.5 TRAINING

1.5.1 GENERAL

All repository staff should be adequately trained to perform the tasks required by their particular position description. Proper training is important to ensure quality in specimen handling. Training should be documented and repeated on an annual base. SOPs should be easily accessible for staff members and should be routinely reviewed for revision. Particular positions related to operating and providing maintenance for specific machinery and equipment (*e.g.*, positions of cryo-engineer and cryo-technician) may require professional education and diplomas/certificates should be assessed. In some areas of safety, adequate training may be mandated by national/federal regulations and severe penalties may be imposed on the repository and repository personnel if training is not provided as required. See *ISBER Best Practices: Recommendations for Repositories 4th edition, Section G: Training,* for details regarding training programs and documentation.

1.5.2 SAFETY

All LN_2 users must be made aware of the properties and hazards and be fully trained in the local repository procedures for usage, storage, and transportation before they engage in handling the substance. Appropriate training in the safe handling of cryogenics should be provided and included in an SOP describing the potential health hazards and required safety precautions and should include training on Safety Data Sheets (SDS).

1.5.3 ALARM RESPONSE

Personnel should be trained on responding to alarms and procedures for recording alarm events and their resolution. Proficiency testing should also be included to determine that personnel know the proper manner by which to respond to alarms.

1.5.4 SPECIMEN RETRIEVAL

Personnel should be trained to take precautions when retrieving specimens from cryostorage. Specimens cryopreserved in LN_2 present a risk of explosion, especially if stored (deliberately or accidentally) in the liquid phase of this medium. Risk of explosion happens when liquid nitrogen enters a storage vial through the cap's screw thread or via microchannels in an improperly heat-sealed glass vial. When the vials are brought to a room temperature environment, the nitrogen expands rapidly, with potentially explosive consequences.

Best Practice: Training should be periodic and documented and in accordance with the needs of the particular tasks to be performed.

1.6 QUALITY CONTROL AND AUDITING

1.6.1 QUALITY MANAGEMENT

Quality Management is a continuous endeavor to improve and standardize management processes and technical procedures of biorepositories to ensure comparable and high quality specimens. A *Quality Management System* (QMS) that includes *Quality Assurance* (QA) and *Quality Control* (QC) programs should cover the full spectrum of a repository's operations. The implementation and maintenance of a QMS contributes to long-term sustainability of repositories. See *ISBER Best Practices: Recommendations for Repositories 4th edition, Section D* for additional details.

Best Practice: Each repository should have a QMS or adhere to the QA program of the organization which the repository is associated with. The program should define the repository's commitment to its QA and QC programs and describe approaches to ensure that the requirements of these programs are met.

1.6.1.1 Quality Assurance

General aspects on QMS, Best Practices, and recommended national and international quality standards and information on non-conformities and Corrective Action and Preventive Actions (CAPA) are provided in detail by the *ISBER Best Practices: Recommendations for Repositories 4th edition, Section D*; however, some specific aspects on LN_2-based storage systems are mentioned below.

1.6.1.2 Performance Testing

Continuous monitoring of the technical infrastructure to avoid any infrastructural failure (*e.g.*, a loss of vacuum in the storage unit) is indispensable and should be governed by respective SOPs. Service and maintenance should be conducted on a routine basis (*e.g.*, annually). The traceability of test equipment as well as the statements for uncertainty of measurement and/or the confidence of conformity statements should be documented. Documents should be audited at least once a year.

Best Practice: Staff members should be properly trained on monitoring LN_2-based storage units and addressing alarms and proficiency with these skills should be tested on a regular basis.

1.6.1.2.1 LN_2-based Storage Units

In addition to the integrated monitoring device for the monitoring of temperature, LN_2 level, and filling time of the LN_2-based storage system, general functionality of the equipment should be controlled regularly by visual inspection and functional tests. Both should be clearly described in an SOP and results should be documented using a defined template form or equipment logbook. This documentation should include, but is not limited to, the date and time, result of the visual inspection and the functional test, and any related measurement.

A comprehensive function test should be performed on the LN_2-based storage unit as well as all auxiliary systems on a regular basis following manufacturer recommendations. Performance testing should include:

- Visual Inspection – units should be inspected for excessive ice or condensation which could indicate a developing vacuum insulation issue that may require corrective action.
- Temperature – the integrated temperature monitoring should be routinely checked and manually verified for accuracy using an independent temperature sensor. Temperature sensors should be calibrated based on manufacturer recommendations. Temperature alarms should be routinely tested for proper function and response.
- LN_2 Level and Usage – should be routinely checked and manually verified for accuracy. The LN_2 usage should be monitored and compared to manufacturer specifications. An increase in LN_2 usage can be the first indication of a potential vacuum insulation issue and may require corrective action. A sudden increase in LN_2 usage that exceeds the planned LN_2 supply capacity could result in temperature excursions and loss of materials.
  - » If the integrated monitoring system provides automatic filling, the process, including component function and user-defined parameters, should be routinely verified. LN_2 level sensors

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should be calibrated based on manufacturer recommendations. LN_2 level and usage alarms should be routinely tested for proper function and response.

- Remote Alarm System – should be routinely tested to ensure the alarm status is correctly communicated to the proper personnel and the established response protocol is followed.
- LN_2 Supply System – should be inspected regularly for excessive ice or condensation which could indicate a developing vacuum insulation issue and may require corrective action. All hoses, valves, and connections should be inspected to ensure they are tight and leak free.
- Oxygen Monitoring – should be routinely calibrated or replaced per manufacturer recommendations. Oxygen alarms should be regularly tested for proper function and response.
- Electricity Supply – primary and backup electricity supply systems should be routinely verified for proper function and switchover. Any electricity supply alarms should be regularly tested for proper function and response.
- Secondary Sensors – any secondary temperature or LN_2 level sensors should be routinely verified and calibrated based on manufacturer recommendations. Any secondary sensor alarms should be regularly tested for proper function and response.

Best Practice: Visual inspections and function tests should be performed on a regular basis. A respective SOP should clearly define all steps of the inspection and the functional tests. Any *deviation* should be documented in writing and reported to the repository manager immediately. Template forms for documentation should be available and regular audits of these records should be performed.

Best Practice: LN_2 usage should be monitored and compared to the manufacturer specifications. An increase in LN_2 usage can be the first indication of a potential vacuum insulation issue resulting in temperature excursions and may require corrective action.

Best Practice: Staff members should be trained accordingly on an annual basis and with any update or change to the process. Training of staff members should be documented and maintained in the employee's training file.

1.6.1.2.2 Alarm Management and Response

In general, clearly structured SOPs should be available that describe actions for every possible alarm that might occur related to LN_2-based storage systems. SOPs should be easily accessible for staff members and should be routinely reviewed for revision. SOPs should cover, but are not limited to, alarms pertaining to LN_2 supply, storage unit temperature, liquid nitrogen levels, filling time, lack of oxygen, and electricity supply. Any alarm or deviation and resultant actions should be documented in written form and reported to the repository manager immediately.

Steps for testing of the functionality of established alarm systems and frequency of testing should be included in an SOP. Functionality should be tested by inducing defined failures. Test results should be documented on a respective form and deviations should be reported immediately to the repository manager to discuss further measures. Functionality of alarm systems should be checked regularly but vary depending on the type of equipment (*e.g.*, alarm pertaining to electricity supply might be tested once a month, alarms pertaining to LN_2 levels tested once a year).

Staff members should be trained according to the specific requirements of alarms and how to test the alarm system. This training should be documented and repeated on an annual basis. It is recommended to attach laminated protocols or checklists to the device to facilitate a direct management of the respective alarm. These protocols or checklists should be a fully integrated part of an SOP on alarm management and should be revised accordingly. Protocols should include, but not be limited to:

- Checking the outside of the unit for condensation indicating that the storage unit vacuum has failed.
- Manually checking liquid nitrogen levels.

- Manually checking temperature inside the unit.
- Checking supply lines or valves to see if they are functioning correctly.

It is recommended to discuss the content of such protocols with the equipment supplier to fully cover all operational aspects of equipment.

Many commercially-available LN_2-based storage units have an integrated monitoring system and data logging device for the record of alarms. The logged data should be copied to a network server regularly and memory capacity of the monitoring system reset.

Best Practice: An alarm chain should be established to ensure that responsible staff members are informed about any possible alarm. Types of alarms, how to manage them, and responsible staff members should be described in a respective SOP. The alarm chain should be managed by an appropriate technical solution or should include an emergency control center to ensure that alarms get passed on to the responsible staff members.

1.6.2 AUDITING

Audits may be done on a quarterly, semi-annual, or annual basis or in response to a non-compliance *incident*, accident, or a change/deviation in procedure required in the light of new information or alterations to ethical, regulatory, or health and safety issues. Internal audits should include the use of an audit checklist and be performed by appropriate and sufficiently trained auditors. Regular audits are primarily directed at prevention of non-conformities as well as detection, corrective action, and process improvement implementation.

Additional external audits may help to further improve proficiency and will foster harmonization of basic processes between repositories. External audits are a routine requirement when establishing a QMS according to the most relevant guidelines and norms (*e.g.*, ISO/DIS 20387:2018, ISO 9001:2015, ISO/IEC 17025:2018, ISO/IEC 15189:2007) or other relevant standards (*e.g.*, AABB, CLSI, CEN, FACT, FAO).

Pertaining to LN_2-based storage systems, internal and external audits may include, but are not limited to:

- Validity of applicable Standard Operation Procedures and Record Template Forms
- Performance of Alarm Management
- Performance of Functional Tests (storage systems, alarm systems, power supply, etc.)
- Documentation of Safety Instructions and Training (hygiene plan, operating instruction)
- Documentation of Functional Tests
- Documentation of Alarms
- Documentation of Maintenance of Alarm Systems and Safety Equipment
- Deviation Management
- Documentation of Deviations

Best Practice: To maintain and increase quality, internal and external audits should be performed on a regular basis. It is recommended to implement a quality management system in accordance with an international standard (*e.g.*, ISO/DIS 20387:2018, ISO 9001:2015, ISO/IEC 17025:2018, ISO/IEC 15189:2007) or other relevant standards (*e.g.*, AABB, CLSI, CEN, FACT, FAO).

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1.7 RELOCATIONS AND TRANSFERS

There are circumstances that require repositories to relocate equipment (*e.g.*, within a facility, from building-to-building) or an entire repository. Since many considerations must be made to ensure an orderly transfer of equipment and supplies, planning should begin as early as possible to ensure effective transfer. In preparation for an unanticipated emergency, a plan should be in place and tested in advance to ensure successful implementation (see *Section 1.8 Disaster Planning/Risk Mitigation*). Consideration should be given to whether the repository has sufficient staff and resources to conduct the relocation independently or if a commercial vendor providing such services should be utilized. See *ISBER Best Practices: Recommendations for Repositories 4th edition, Section B.10: Relocation of a Repository* for additional information.

Best Practice: A map should be created for the new site that will indicate the location of all equipment and materials that will be transferred prior to the initiation of the move.

Best Practice: Planning should include review of current processes to ensure that they can be efficiently implemented into the new space.

1.7.1 SPECIMEN INTEGRITY

Handling during removal from storage will affect the viability of cells and may result in degradation of cellular components. Every time a specimen is warmed above the *Tg* it experiences a micro-thaw event. Repeated thermal cycling episodes lead to increased cell death via apoptosis and necrosis. The temporal nature of delayed onset cell death resulting from preservation stress may affect the quality of data obtained from these specimens depending on the timing of experiments post-preservation and the ability of the cells to recover from cryoinjury in the long-term. For these reasons it is essential to limit the potential of cooling/re-warming, freeze/thaw, and *vitrification*/devitrification cycles occurring when specimens are relocated or transferred.

1.7.2 PLANNED MOVES

While *distribution* and shipping of cryogenic specimens or even sets of specimens is a routine practice, larger transfers or complete relocations can present some significant challenges. Depending on the number of units being moved, several strategies may be employed. If specimens are to be transferred from an existing facility to another existing facility already prepared to receive cryopreserved specimens, specimens must be packaged, shipped, received, accessioned, and placed into storage all while maintaining temperature. Prior to any move of this type, transient warming tolerance levels for the specimen prior to engaging in the process of shipping (if not already known) should be evaluated.

A thorough Move Plan should be developed when a repository is being moved from one location to another. Additional considerations surrounding equipment relocation must be addressed while the same consideration regarding specimen identification and temperature maintenance still must be managed. For shipping large numbers of cryopreserved specimens, large, palletized *liquid nitrogen (LN_2) dry shippers* should be used that are easily moved with a pallet-jack or forklift. A palletized dry shipper must have means to lock the inventory in place during transit. Portable workbenches may facilitate transfers by allowing re-organization or tracking of inventory as it is moved in bulk from the primary storage unit to the shipper.

Temperature monitoring should be performed during the duration of the shipment. Because LN_2 shippers rely on nitrogen for cooling, on-truck generators are not necessary; however, a truck that can accommodate backup nitrogen is required for long moves by ground. For air freight, local and international law must be observed and may have more restrictions than ground transport. Groups such as IATA (International Air Transport Association) may be consulted to ensure appropriate measures are being taken.

Best Practice: To minimize risk of loss, consider splitting duplicate specimens between dry shipping units and shipping in separate shipments.

Best Practice: When shipping specimens in aluminum transportation Dewars, utilize protective *containers* that minimally meet the ISTA-3A qualification for the combined Dewar and protective container to reduce risk of loss.

1.7.3 QUALIFICATION PROCESSES

Once the LN_2 storage unit is emptied, it should be decontaminated as per internal protocol and freight-shipped to the new site. It may be shipped with the dry shipper or on its own, depending on logistical benefit. Upon receipt at the new facility, IQ must include a thorough inspection for damage due to transport. A similar OQ/PQ should be performed as per when the equipment was originally installed to ensure the system will still be suitable. If separate equipment validation procedures are performed beyond PQ, these may be abbreviated.

The dry shipper should be received on or about the same time as the emptied LN_2 storage unit and can be maintained at temperature while appropriate IQ/OQ/PQ is performed on the original LN_2 storage unit. Upon completion of qualification, specimens may be re-introduced to the original LN_2 storage unit and the shipper can be sent back for the next *batch*. The number of LN_2 storage units sent at any one time is a function of staff and expense; however, careful planning and organization is required to coordinate. The plan should ensure that both inventory location and temperature is known throughout the entire process.

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1.8 DISASTER PLANNING/RISK MITIGATION

An emergency response plan should be a component of the disaster recovery plan. Emergencies can cover a wide range of natural and man-made disasters, all of which may have varying effects on the facility and on the ability of the repository to carry out its essential functions. The type and duration of disasters may depend on the geographic location at which the repository is located. Depending on the "value" and the ability to replace certain specimens, some repositories may decide to divide *collections* and store them in different environmental storage units or even at different geographic locations so that a disaster affecting one component of the collection would not eliminate the entire collection.

Repositories should have a written disaster recovery/*incident* response and business continuity plan for responding to a wide variety of emergency situations. This plan should be tested periodically (*e.g.,* at least annually) to ensure that all personnel are trained and that the plan meets the anticipated needs. Copies of these plans should be distributed to all appropriate staff.

Best Practice: The Director or appropriate staff member should communicate with local power providers before an emergency occurs to request that the repository be placed on a list of "high priority" users for power restoration following an emergency and prepare a list of reasons for this requirement.

Best Practice: Notification of security and *environmental monitoring systems* should be verified on a routine basis. Where possible, emergencies should be simulated to ensure proper follow-through for the established emergency plan.

Best Practice: If repository inventory systems are housed on a server located away from the repository, some consideration should be given to storing electronic inventory records on site to ensure that needed records are accessible in an emergency.

Best Practice: In the event of an impending need for evacuation, repositories that utilize LN_2 bulk tank(s) should arrange to have them filled.

Best Practice: Duplication of specimen collections and data in distinct locations (*e.g.*, including in different LN_2 storage units) is recommended to ensure preservation of the holding in the event of a catastrophic event.

1.8.1 ON-CALL STAFF

Key individuals should be identified who will serve as being "on call" or who will be able to respond to an emergency at the repository. These individuals should be properly trained and qualified to address all situations associated with LN_2-based specimen storage. Leave and vacation schedules should be monitored to ensure that coverage of essential responsibilities is in place should key individuals be unavailable. Emergency contact numbers should be posted in prominent locations in the repository and should be carried by staff members at all times who are "on call". The contact information should be reviewed on a regular basis to ensure that the information contained therein is current.

Best Practice: Repositories should have a check list of activities for "on call" staff to follow during an emergency. "On call" staff should be familiar with the location and operation of certain key equipment and controls (*i.e.,* circuit boards) that may need to be checked during an emergency. Telephone numbers for professional assistance should be clearly posted in the repository and accompanying administrative areas (*e.g.*, engineering or facilities personnel, power companies, fuel supply companies, transportation services).

Best Practice: The on-call system should be routinely tested to ensure it is functioning properly.

1.8.2. BACKUP CAPACITY

Adequate backup capacity for low temperature units should be maintained in anticipation of possible equipment failure. If space and funds allow, backup storage for each storage condition should be available within the repository. Where this is not possible, repository staff should identify backup space in a nearby facility to allow for transfer of specimens in case of an emergency. When *co-location* is not possible, LN_2, dry ice, and/or portable freezers should be available at the facility to maintain specimens during transfer to the backup units off-site in the event of an emergency. Additional backup power calculated to sufficiently cover power consumption of storage equipment, monitoring equipment, and key auxiliary equipment (*e.g.*, air conditioners, matching LN_2 storage units) should also be available. Refer to *ISBER Best Practices: Recommendations for Repositories 4th edition, Section C.9: Backup Storage Capacity* for additional information.

Best Practice: Extra-capacity equipment should be equal to the capacity of the largest single LN_2 storage unit and should be maintained in reserve at operating temperature. The total amount of backup storage required for large repositories should be determined empirically.

Best Practice: Repositories should have a written procedure for transferring specimens from a failed or malfunctioning unit (one that has exceeded or is on the verge of exceeding its acceptable operating temperature range or become over-filled) and for the return of the specimens to their original location once it is considered safe to do so. The procedure should include the LN_2 storage unit name or number as well as the location within the LN_2 storage unit where the specimens have been relocated.

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1.9 STORAGE CONTAINERS AND LABELS

For cryogenic storage of specimens, *biobanks* and repositories may utilize mechanical freezers, nitrogen, or vapor nitrogen for storage to maintain cryogenic temperature (*e.g.*, <-130°C). The container system, the suitability of the materials for long-term low temperature storage, and biocompatibility are all critically important; especially for non-fixed, liquid specimens such as blood, other cell types, or bodily fluids. Only packaging and storage containers and labels designed to withstand cryogenic temperatures should be used. For specimens in storage for clinical utilization, these issues take on additional safety importance. Therefore, an ideal cryopreservation container system must provide for:

- Type of Container – A container needs to be chosen based on fit-for-purpose taking product type and volume into consideration.
- Material Suitability – The system must remain mechanical integrity and be stable over long periods of time as well as be biocompatible.
- Closure Integrity – The system needs to be hermetically sealed to ensure integrity of the specimen is maintained throughout *processing*, storage, and distribution.

Like water, LN_2 can act as a vehicle for the transmission of viruses, bacteria, fungi, and animal cells and should be treated as a *biohazard*[5]. Viruses have previously been found to survive direct exposure to liquid nitrogen, including vesicular stomatitits virus[5], herpes simplex virus, adenovirus[6], and papilloma virus[7], and a hepatitis B virus outbreak due to cross contamination under liquid has been recorded[8]. There is also evidence of contamination of liquid nitrogen by other microorganisms, including a wide range of bacterial and fungal species[9,10,11,12].

Additionally, as therapeutic cell products reach later stages of clinical trials, more consideration is being placed on manufacturing processes that are capable of meeting regulatory requirements for good manufacturing practices (GMP) and producing commercial-scale quantities of living cell products. Attention is being paid to the large-scale production of therapeutic cells[13], with downstream processing and fill-finish operations being cited as bottlenecks in current and future cell manufacturing[14].

1.9.1 CONTAINER TYPES

1.9.1.1 Vials

Currently, many cell-based products are stored in screw-cap vials, technology utilized for decades in research laboratories and sperm banks. Screw-cap vials have been used due to the relative ease and convenience of adding and removing specimens, despite obvious drawbacks to the container closure integrity of these systems. Many screw-cap vial configurations are available with similar diameters ranging in size from 0.2 to 5 mL volumes. The submersion of screw-capped plastic vials allows for contact between contaminated LN_2 and the specimen. Screw-cap vials may have internal or external threads, neither of which should be stored in the liquid phase of nitrogen but are validated for vapor phase.

Commercial-scale *lot* sizes of hundreds to thousands of living cell doses per lot required to supply commercial-scale cellular products benefit from utilizing technologies previously developed for pharmaceutical production, including large fill-finish vialing systems. As large scale production of therapeutic cells becomes a reality, downstream processing is being cited as a significant bottleneck in current and future cell manufacturing[14].

1.9.1.2 Bags

Cell freezing bags, a technology borrowed from the blood banking industry, have been the container of choice for most cell therapy biopreservation due to the available infrastructure for processing, freezing, and storage of these container systems. Blood bags are available in a wide range of volumes, with most utility being in volumes larger than 5 mL. While smaller-volume bags are available, complete specimen withdraw can become challenging. Cell freezing bags can be configured in any number of ways with off-the-shelf ports and tubing sets, allowing flexibility to customize around filling and retrieval processes. These bags are also available with unique serial numbers which are reproduced on integral tubing to allow test segment production and identification for future testing of the product.

Choice of container is inherently based on the type of specimen, the utilization purpose, and the volume required. It is recommended to use vial systems for volumes <5 mL to facilitate recovery of the full specimen and to use bags for larger volumes and use bags with serialized test segments if specimens that are integral to the unit are required for future testing.

1.9.1.3 Cryogenic Straws

Cryogenic straws are hermetically sealed and specifically designed for the safe storage of specimens in the liquid phase of nitrogen. These straws should be made of a material that is chemically inert, biocompatible, and have physical characteristics that make them resistant to ultra-low temperatures and pressures created by their storage conditions. They are stable when submitted to sudden low temperatures (snap-freezing), when held at low temperatures for long periods of time (years), or when taken through several freeze-thaw cycles.

1.9.2 MATERIALS SUITABILITY

Generally, cryopreservation containers should be constructed of materials chosen specifically for their resistance to chemicals, drainability for maximum cell recovery, and durability under cryogenic temperatures. If the cryopreserved product is contemplated for medical use, the container must also be constructed using USP class VI materials.

Screw-cap cryovials are generally made of polypropylene, with various additional resins utilized for the cap and/or threads to enhance stability at temperature, such as polyethylene. Polypropylene is a plastic resin that has been used for decades in various packaging applications, including bottles, pouches, tubes, and containers. However, these plastic resins have been rarely used in parenteral pharmaceutical production for vials due to various quality[15].

The availability of newer plastic resins such as cyclic olefin co-polymers has allowed development of new vials systems with key features such as glass-like clarity, lower extractables, ability for various modes of sterilization, very low moisture permeability, biocompatibility, and lower particulates and has enabled the use of these plastics for pharmaceutical, biopharmaceutical, and cell therapy storage and delivery applications. Cyclic olefin co-polymer (COC) resins are currently being used to package and deliver pharmaceutical drugs worldwide[16,17].

Cell freezing bags have historically been constructed with ethylene vinyl acetate (EVA) and polyvinyl chloride (PVC). EVA experiences a glass transition temperature normally beginning at -15 °C, making its use questionable in environments below -15 °C[18]. This thermal transition of EVA makes freezing bags extremely brittle at temperatures at which cell suspensions are typically stored. This results in the cell freezing bags being fragile and susceptible to breakage throughout cryopreservation processing and storage. This period of vulnerability includes the long-term storage of the cellular product, which can be measured in decades. Any failure of the cell freezing bag in this interval of vulnerability will likely lead to the contamination of the contents and in turn can cross-contaminate other products stored in the same LN_2 storage unit[9,19]. Another significant concern with many cell freezing bags is the extensive use of PVC tubing for filling and retrieving specimen from the bags. PVC tubing becomes brittle at typical cell suspension storage temperatures and will snap and easily break with even minimal force. Further, the plasticizers used in making PVC materials flexible are known to leach out of the plastic and into the surrounding media.

Newer generations of cell freezing bags are constructed with fluorinated ethylene propylene (FEP). This material exhibits extreme temperature resistance while maintaining dimensional stability and strength. Studies comparing EVA to FEP durability through temperature excursions and drop-testing have demonstrated the suitability of FEP over EVA[20].

1.9.3 CLOSURE INTEGRITY

Ideally for any cryostorage, and necessary for clinically banked specimens, the container must have a closure system that can be hermetically sealed and remain stable through cryopreservation and biobanking procedures[21,22]. Similar to water, LN_2 can act as a vehicle for the transmission of viruses, bacteria, fungi, and even animal cells and it has been suggested that LN_2 exposed to viruses should be treated as a biohazard[5]. The submersion of screw-capped plastic vials and open gamete/embryo vitrification devices allows for contact between contaminated LN_2 and the specimen. At temperature, condensation of the atmosphere within the vial creates a vacuum which can draw in LN_2 and any contaminants in the LN_2 may contaminate the specimen. A previously published study indicated 45% of cryovials without an O-ring gasket and 85% of vials with an O-ring absorbed LN_2 during 3 hours of immersion[23].

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Until recently, bag storage (despite the potential for failure due to breakage) remained the most secure method to maintain cryostored cells in a closed system. While screw-cap vials have been used for decades for smaller volumes, tests have routinely demonstrated clear risks using these systems. One potential way to mitigate LN_2 ingress is to store in vapor-phase nitrogen. Threaded vials should be used in vapor phase storage only; however, breakage may still occur as typical containers become brittle even at vapor phase temperatures and viruses can potentially remain air-borne and still coat adjacent containers. Several vial systems are now available that meet container closure standards, including those with bag-style tubing systems, laser-welded stoppers, and low-temperature stoppered vials.

Cell freezer bags are typically closed by RF welding the tubing, which provides a robust seal. Overwraps are typically available for most cell freezer bags which allow an additional layer of protection; however, this must be taken into account while developing cooling and warming protocols as they add some insulation. Integral test segments associated with bags and some vials, while necessary for many clinical processes, present a potential failure point when detaching at cryogenic temperatures and subject the primary specimen to potential transient warming events. If segments are serialized with unique identification numbers matched to the primary container, it is recommended that they be detached and cryopreserved and stored separately.

Best Practice: The container of choice for a specific processing, storage, and shipping regimen should be validated with respect to container closure integrity. This includes drop testing from 1 m directly from cryogenic freezing as well as simulated shipments.

1.9.4 LABELS

Each specimen should receive a label with a strong, permanent adhesive that can withstand cryogenic temperatures and survive all planned processing conditions. Information printed on labels should be resistant to all common laboratory solvents. Labels should contain an ID linking to a database containing details about the specimen collection and processing information. See *ISBER Best Practices: Recommendations for Repositories 4th edition, Section I2.2: Specimen Descriptors* for additional guidance.

Best Practice: The adherence of labels to containers as well as the use of particular types of ink should be tested before they are put into regular use.

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APPENDIX A: INTERNET RESOURCES

These internet references are made available for information only. ISBER does not warrant any of the information contained therein.

Subject	Website	Organization	Topics
Best Practices for Biological Resource Centers	http://www.oecd.org/dataoecd/7/13/38777417.pdf	Organisation for Economic Co-Operation and Development	Consensus Best Practices for Biological Resource Centers in OECD Countries
Biorepository Protocols	http://www.abrn.net/protocols.htm	Australasian Biospecimen Network	Protocols and best practices for collecting and processing human biospecimens
Biosafety	http://governance.iarc.fr/ENG/Docs/safetymanual.pdf	The Division of Biosafety and Biotechnology (SBB), Scientific Institute of Public Health in Belgium.	Biosafety risk assessment tools and biosafety manuals, laws, and regulations; guidelines on containment facilities, equipment, and practices; shipping and transport
Biosafety	http://www.ebsaweb.eu/Resources.html	European Biosafety Association	Conferences and other resources on European biosafety issues
Biosafety	http://www.ebsaweb.eu/ebsa_media/Downloads/Biosafety7-view_image-1-called_by-ebsa.pdf	World Health Organisation	Laboratory biosafety manual covering equipment and facility design and techniques
Biosafety	http://www.cjd.ed.ac.uk	UK Surveillance Unit for Creutzfeldt-Jakob Disease	Surveillance data on Creutzfeldt-Jakob Disease; technical information; links
Biosafety	https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines.html	Government of Canada	Standards and guidelines for the safe handling of human and animal pathogens, toxins, and plant pests in laboratories and containment zones
Case Studies of Human Tissue Repositories	http://www.rand.org/pubs/monographs/2004/RAND_MG120.pdf	Rand Corporation and the National Cancer Institute	Best Practices for repositories based on information collected from a defined number of U.S.-based repositories
Chemical and Laboratory Resources	http://www.neis.com/environmental_resources.html	Chemindustry.com	A variety of resources for laboratory equipment and supplies for over a hundred countries world-wide (under the tab for "lab supplies")
Chemical Safety	http://www.cdc.gov/niosh/database.html	National Institute for Occupational Safety and Health (NIOSH), U.S.	Databases and information resource links and publications in the United States
Chemical Safety	http://www.ilo.org/public/english/protection/safework/cis/products/icsc/dtasht/index.htm	International Occupational Safety and Health Information Center	Chemical database; International Chemical Safety Cards (ICSC)
Chemical Safety	http://www.cdc.gov/niosh/chem-inx.html	Master Index of Occupational Health Guidelines for Chemical Hazards (NIOSH), U.S.	U.S. National guidelines for chemical hazards of specific chemicals
Chemical Safety	http://www.who.int/ifcs/en/	Intergovernmental Forum for Global Chemical Safety	Policy guidance on chemical safety
Chemicals Management	http://www.environment.gov.au/settlements/chemicals/index.html	The Australian Government Department of the Environment and Water Resources	Chemicals management strategies to protect human health and the environment
Electrical Safety	http://www.ehs.uconn.edu/Word%20Docs/Electrical%20Safety%20in%20the%20Lab.pdf	University of Connecticut Environmental Health and Safety	Electrical safety in the laboratory
Environmental Specimen Bank Design	http://www.ehponline.org/members/1995/Suppl-3/wise-full.html	U.S. National Institute of Standards and Technology	Paper presented at the Conference on Human Tissue Monitoring and Specimen Banking: Opportunities for Exposure Assessment, Risk Assessment, and Epidemiologic Research held 30 March-1 April 1993 in Research Triangle Park, North Carolina

ISBER BEST PRACTICES

Subject	Website	Organization	Topics
Exposure Prevention Program Information	http://www.healthsystem.virginia.edu/internet/epinet/subpage2.cfm	Exposure Prevention Information Network; University of Virginia, International Health Care Worker Safety Center	Provides standardized methods for recording and tracking percutaneous injuries and blood and body fluid contacts
Genebank Standards for Plant Genetic Resources for Food and Agriculture	http://www.fao.org/3/a-i3704e.pdf	Food and Agriculture Organization of the United Nations (FAO)	General and technical information for the organization and management of biobanks of plant genetic resources for food and agriculture
General Safety	http://www.osha.gov/comp-links.html	Occupational Safety and Health Administration, Department of Labor, USA	Current U.S. regulations and regulations under development; technical, prevention, and training information; links
General Safety	http://www.lbl.gov/ehs/pub3000	Lawrence Berkeley National Laboratory; University of California, California, U.S.	U.S.-based health and safety manual
Laboratory Automation	http://www.slas.org/	Society for Laboratory Automation and Screening	Automation in the laboratory including liquid handling, sample storage and retrieval, specimen processing
Laboratory Standards Development	http://www.clsi.org	Clinical and Laboratory Standards Institute	U.S.-based general and technical Information for the development of laboratory standards
National Cancer Institute Best Practices for Biospecimen Resources	http://biospecimens.cancer.gov/NCI_Best_Practices_060507.pdf	National Cancer Institute; National Institutes of Health; U.S. Department of Health and Human Services	Best Practices for biospecimen handling, processing, storage, and retrieval for specimens collected through NCI-sponsored research
Occupational Health and Safety	http://governance.iarc.fr/ENG/Docs/safetymanual.pdf	International Agency for Research on Cancer	Health and safety manual
Occupational Health and Safety	http://www.ccohs.ca/	Canadian Centre for Occupational Health and Safety	Information on biological hazards, chemical and materials, health and safety programs
Resources for Pathology Laboratories	http://www.cap.org/apps/cap.portal?_nfpb=true&_pageLabel=reference	College of American Pathologists	General and technical Information for lab management for U.S.-based laboratories

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APPENDIX B: GLOSSARY

Unless otherwise defined in another context in these Practices, important terms are defined below.

AUDIT – A documented review of procedures, records, personnel functions, equipment materials, facilities, and/or vendors in order to evaluate adherence to written standard operating procedures or government laws and regulations.

BATCH – A specific quantity of specimens that is intended to have a uniform character and quality, within specific limits, and is produced or processed according to a single processing protocol during the same processing cycle. (see LOT).

BIOBANK – See REPOSITORY.

BIOHAZARD – An organism, or substance derived from an organism, that poses a threat to (primarily) human health. This can include medical waste, samples of a microorganism, or virus, or toxin (from a biological source) that can impact human health. It can also include substances harmful to animals.

BIOREPOSITORY – See REPOSITORY.

CALIBRATION –The process of adjusting the output or indication on a measurement instrument to agree with value of the applied standard, within a specified accuracy.

COLLECTION – May refer to the practice or technique of collecting a specimen (See RETRIEVAL) or to a specific sample or group of samples that has been isolated for future research purposes.

CO-LOCATION – To share a location.

CONTAINER – Enclosure for one unit or more units of specimen(s).

DEVIATION – An intentional or unintentional event that is a departure from a procedure or a normal practice.

DEWAR – A specialized container to hold liquefied gases. A Dewar may also be referred to as a Dewar flask, Dewar vessel, or Dewar tank.

DISTRIBUTION – A process that includes receipt of request for specimens, selection of appropriate specimens, and final inspection, in conjunction with subsequent shipment and delivery of specimens to another repository, specimen collection center, or laboratory.

DRY ICE – Solid phase carbon dioxide (CO_2). CO_2 solidifies at -78.5 °C.

ENVIRONMENTAL MONITORING SYSTEM – An automated, centralized monitoring system that monitors environmental conditions and alarms in conjunction with remote access, security features, and electronic data storage.

EUTECTIC TEMPERATURE – The temperature at which all of the components of a mixture crystallize simultaneously.

GLASS TRANSITION TEMPERATURE (Tg) – The glass transition temperature marks the temperature at which a fluid becomes so viscous it appears solid. The extreme viscosity reduces diffusion and molecular restructuring, slowing reactions that might otherwise cause samples to deteriorate. The *Tg* for pure water is -132°C.

INCIDENT – Any unplanned occurrence that deviates from SOPs or applicable government laws and regulations during specimen retrieval, processing, labeling, storage, or distribution that may affect subsequent use of those specimens.

LIQUID NITROGEN (LN_2) – Coolant used to cool and store samples. Nitrogen becomes liquid at -196°C. Samples stored in the vapor phase of liquid nitrogen are -190°C and warmer, depending on the distance from the liquid phase.

LIQUID NITROGEN DRY SHIPPER– A container used for sending samples in the vapor phase of liquid nitrogen.

LIQUID NITROGEN (LN_2)-BASED STORAGE UNIT – A double-walled, vacuum insulated unit designed to efficiently hold LN_2 for the storage of clinical and research specimens. Also referred to as liquid nitrogen freezer, vessel, or tank.

LOT – A quantity of reagents, supplies, or containers that is processed or manufactured at one time and identified by a unique identification number (see BATCH).

PRESERVATION – Use of chemical agents, alterations in environmental conditions, or other means during processing and storage to prevent or retard biological or physical deterioration of a specimen.

PROCEDURE – A series of steps designed to result in a specific outcome when followed in order.

PROCESSING – Any procedure employed after specimen collection but prior to its distribution, including preparation, testing, and releasing the specimen to inventory and labeling.

QUALITY – Conformance of a specimen or process with pre-established specifications or standards.

QUALITY ASSURANCE (QA) – An independent integrated system of management activities involving planning, implementation, documentation, assessment, improvement, and audit to ensure that a process or item is of the type and quality needed for the project.

QUALITY CONTROL (QC) – Specific tests defined by the QA or QMS Program to be performed to monitor procurement, processing, preservation, and storage; specimen quality; and test accuracy. These may include but are not limited to: performance evaluations, testing, and controls used to determine accuracy and reliability of the repository's equipment and operational procedures as well as monitoring of the supplies, reagents, equipment, and facilities.

QUALITY MANAGEMENT SYSTEM (QMS) – a documented system to improve and standardize management processes and technical procedures of repositories to ensure comparable and high quality specimens. Includes Quality Assurance (QA) and Quality Control (QC) programs that cover the full spectrum of a repository's operations.

REPOSITORY – An entity that receives, stores, processes, and/or distributes specimens, as needed. It encompasses the physical location as well as the full range of activities associated with its operation. It may also be referred to as a BIOREPOSITORY or BIOBANK.

RETRIEVAL – The removal, acquisition, recovery, harvesting, or collection of specimens.

SAFETY – Processes, procedures, and technologies to ensure freedom from danger or harm.

SAMPLE – A single unit containing material derived from one specimen. A portion of a whole.

SPECIMEN – A specific tissue, blood sample, etc., taken from a single subject or donor at a specific time. For some biological collections "specimen" may have the same meaning as "individual."

STORAGE – Maintenance of specimens under specified conditions for future use.

TELEMETRY SYSTEM – A system that allows for measurements to be taken from a distance, usually via radio wave transmission and reception of the information.

VITRIFICATION – Refers to the transformation of a glass-forming liquid into a glass, which usually occurs upon rapid cooling. It is a dynamic phenomenon occurring between two distinct states of matter (liquid and glass), each with different physical properties.

APPENDIX C: ABBREVIATIONS

Below is a list of abbreviations that are used throughout this document:

AABB – American Association of Blood Banks

CAPA – Corrective Action and Preventive Actions

CLSI – Clinical & Laboratory Standards Institute

CEN – Comité Européen de Normalisation (European Committee for Standardization)

EVA – ethylene vinyl acetate

FACT – Foundation for the Accreditation of Cellular Therapy

FAO – Food and Agriculture Organization

FEP – fluorinated ethylene propylene

IATA – International Air Transport Association

ID – identification reference

IQ – Installation Qualification

ISO – International Organization for Standardization

mL – milliliter

LN_2 – liquid nitrogen

OQ – Operational Qualification

PQ – Performance Qualification

PVC – polyvinyl chloride

SDS –Safety Data Sheet

SOP – Standard Operating Procedure

PHI – Personal Health Information

QA – Quality Assurance

QC – Quality Control

QMS – Quality Management System

SOP – Standard Operating Procedure

Tg – glass transition temperature

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NOTES

ADDENDUM 1:

Liquid Nitrogen-Based Cryogenic Storage of Specimens

INTERNATIONAL SOCIETY FOR BIOLOGICAL AND ENVIRONMENTAL REPOSITORIES

BEST PRACTICES:
Recommendations for Repositories
Fourth Edition

The Liquid Nitrogen-based Cryogenic Storage of Specimens Best Practices Addendum is a collaboration between the International Society for Biological and Environmental Repositories and the Society for Cryobiology that reflects the collective experience and knowledge of industry experts.