Reprinted from *American Biotechnology Laboratory* December 2005

Procurement and Supply of Cell Storage Systems for the Biotechnology Laboratory

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Specifying a cell storage system is an important decision for any life sciences laboratory. A poor choice can put valuable research at risk and add costs. A good choice can reduce aggravation and lower expenditures. Planners need to consider a variety of factors when selecting a cell storage system, including temperature needs, power demands, sample contamination, potential facility requirements, safety, and cryogen supply. This article compares the two types of storage systems—liquid nitrogen (LIN) freezers and mechanical freezers—to help the reader make more informed purchases.

Temperature

The first question that a planner should ask when selecting a storage system is "How cold is cold?" For many applications, cold enough means achieving the glass transition temperature of water (–130 °C). At this temperature, movement within a cell ceases, putting it into a state of suspended animation. This enables the sample to survive for a virtually indefinite period of time.

There are two primary options for storing samples at this temperature: 1) LIN freezers and 2) mechanical freezers. LIN units offer the coldest temperatures. They store samples in a pool of LIN at –196 °C, or, in the case of a vapor phase unit, in cold nitrogen vapor at temperatures of –170 to –190 °C. Mechanical freezers store samples at somewhat warmer temperatures. The coldest mechanical units achieve temperatures of –130 °C to –150 °C, although the vast majority of mechanical freezers are designed to cool to –80 °C.

In general, mechanical units are the more convenient option. The user simply plugs in the unit and sets the temperature to get started. However, to achieve temperatures at or below –130 °C, mechanical units must operate at the very edge of their ranges, making samples somewhat vulnerable to fluctuations in temperature. Consequently, these units are still somewhat rare. In contrast, LIN units are the more common and more reliable option for storage below the glass transition temperature and for long-term sample preservation.

Figure 1 LABS 40K freezer.

Sample integrity

In recent years, the potential for contaminants such as viruses, bacteria, and fungi to migrate from cell to cell when immersed in LIN has become an issue. Laboratories are under pressure to protect samples from contamination. One way for them to do this is to provide additional protective containers around the vials that contain the samples. Another way is to store samples in a vapor phase unit, which keeps the samples below the glass transition temperature, without direct contact with the LIN.

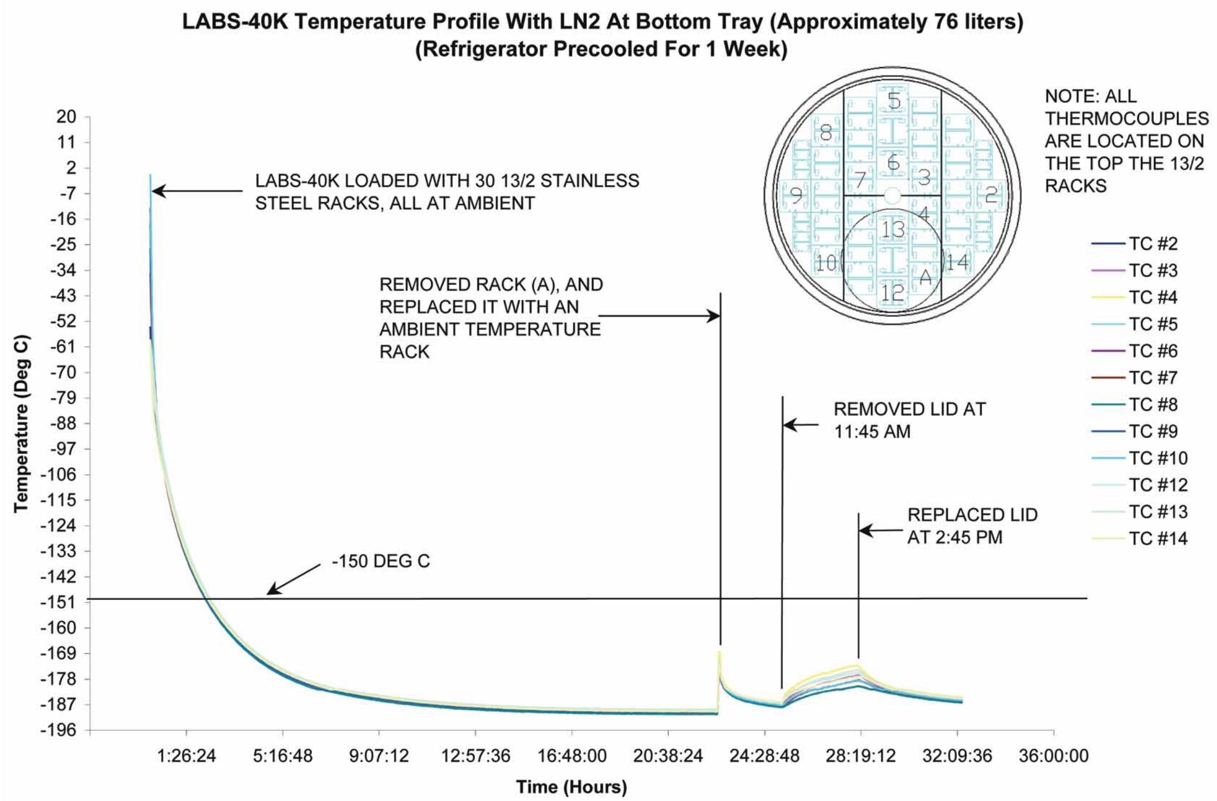

Figure 2 *Temperature of vapor phase unit.*

The argument has been made that LIN vapor phase units have large temperature gradients and therefore provide inconsistent freezing. This may be true for some older units that have large lids. However, newer designs, equipped with improved control systems, maintain extremely consistent temperatures. For example, in a temperature profile test, the LABS 40K™ unit, equipped with a KRYOS® control system (**Taylor-Wharton Cryogenics,** Theodore, AL) (*Figure 1*), demonstrated the ability to maintain a consistent temperature of –180 to –187 °C across the top of the racks. In fact, when the lid was left open for 3 hr, the temperature still held within a range of –171 to –181 °C (see *Figure 2*). It is unlikely that a mechanical unit would perform as well with its door open for this extended period of time.

Storage above the glass transition temperature

Conventional wisdom says that because mechanical freezers are convenient, they are the natural choice for storage above the glass transition temperature. However, the choice is not so simple. Users need to consider a host of factors, some of which may make an LIN freezer the practical choice above the glass transition temperature also. In reality, most facilities use a combination of mechanical and LIN units to meet their needs. Following are some factors to consider when making decisions for specific applications.

Facility planning issues

Facility issues such as power demands, space, location, cleanroom, and secure access requirements all play important roles. Users should consider questions such as, "How much electricity will I need to operate the freezers?" and "How do I supply units on upper floors?" A discussion of these issues follows.

Power

Power consumption is a primary consideration. All storage systems require some electricity to operate. However, mechanical freezers consume much more power to run compressors and refrigeration systems that achieve low temperatures. The electricity to run a mechanical freezer costs approx. $1100–$2200 per year, depending on the local price of electricity. Most facilities have 20–30 freezers; thus the annual power consumption can easily cost $44,000–$66,000. Conversely, an LIN unit consumes less power than a light bulb to run its automated controls. It achieves its low temperatures through cryogens.

Added to this higher power demand is air conditioning the room with mechanical freezers. The compressors on a mechanical unit emit about 3200 BTU of heat per

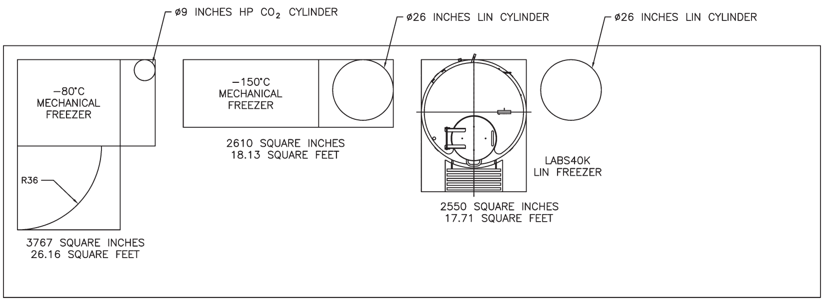

Figure 3 *Unit dimensions.*

Another consideration is that LIN freezers are a good option in places in which headspace is limited. LIN freezers are built chest style—the average LIN unit is a little less than 4.5 ft tall, significantly shorter than a 6.5-ft mechanical freezer. An additional 1.5 ft of headspace should be allowed for the lid to open so that personnel can retrieve samples when necessary.

hour. A room could quickly go from 70 to 100 °F, putting an increased load on the air conditioning system. For one –80 °C unit, this will add $300 per year, depending on local power costs. For the entire facility, this could mean an additional $6000–$9000 power cost. In some older facilities, sufficient air conditioning may not be available, requiring upgrades that may make mechanical freezers cost prohibitive.

Also, the possibility of a power disruption should be considered. During a disaster, or even a more common brownout or blackout, the lack of power can put samples at risk, especially if the proper backup systems are not in place. Mechanical freezers operate much in the same way as household freezers—without power, the unit does not generate cold temperatures. The typical –80 °C mechanical unit therefore uses high-pressure CO_2 cylinders that keep the unit cold for approx. 5–7 hr if the power goes down. One should keep in mind that the gas coming from the cylinder is –50 °C, which is warmer than the intended temperature of a –80 °C freezer. For colder temperatures, an LIN backup cylinder is required. Conversely, a typical LIN freezer deprived of electricity can keep samples below the glass transition temperature for up to 10 days, provided the lid is kept closed. Backup systems will be discussed in detail below in the section on managing the supply chain.

Space

Because space is at a premium in most biotechnology laboratories, planners need to carefully consider their short- and long-term space needs when selecting units. A typical –80 °C freezer, with a capacity of 48,000 vials, takes up a little over 26 ft^2, including the space to open the door and a 6-in. space around the unit for airflow. The unit may require a 9-in.-diam CO_2 cylinder for backup. In contrast, a LABS 40K unit takes about 17 ft^2 to hold 41,500 vials (see *Figure 3*). It must be kept in mind that the LIN unit uses a 26-in.-diam LIN cylinder. If a laboratory chooses to keep the LIN cylinder right next to the unit, the space requirement is about 30 ft^2. However, planners may want to consider implementing alternative supply modes such as an external bulk or microbulk tanks located in less space-constrained areas to conserve space in the freezer room.

Weight and facility limitations

When selecting units for upper floors, weight limitations can restrict the size of the individual storage unit. The evaluation can become very detailed and specific; therefore it is a good idea to review the building codes prior to selecting a unit. Additionally, the physical dimensions of elevators, doorways, and corridors may limit the choice of units for upper floors. For example, a larger unit may be ideal for a first-floor installation but may not work on a second floor if there is not an elevator of suitable size. Likewise, larger units may not fit through narrow doors or corridors.

Validation

Validation is a process that has to be undertaken for both mechanical and LIN units. Validation includes several processes, including installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ)—costing approx. $1000. This cost can increase depending on individual customer specifications and needs to be factored into the cost for each unit. In general, the fewer the units, the lower the validation cost. Thus, where possible, one should consider selecting the largest unit in a series to minimize this cost. For example, a LABS 80K™ unit provides about the same storage capacity of two LABS 40K units at half the validation cost.

Maintenance

Maintenance is another cost that must be considered. Mechanical freezers contain a variety of working parts—compressors, electronic relays, thermostats, pressure sensors, and valves—that can and do fail, requiring maintenance. The worst is a compressor failure, which can cost around $3000 to fix. Typically, the customer needs to keep a spare unit available in the event of such a failure. The replacement unit must be brought up, sterilized, validated, and installed. Samples then have to be moved to the new unit at an additional cost, all before the failed unit can be taken out of service for repair.

In contrast, LIN units have few working parts and require almost no maintenance. The only moving part on an LIN freezer is a solenoid valve, which should be replaced every 3–5 years at a cost of approx. $300. Most repairs can be made on site and without taking the unit out of service. The exception to this is a freezer vacuum failure, which is a very rare event.

Also, the compressors on mechanical units make noise. One unit may not be an issue, but 15 or 20 may be significant. The noise increases as the compressors age—like the compressor on an older kitchen refrigerator—making replacement a more pressing issue, particularly if the freezers are located in work areas.

Cleanrooms and secure access

Maintenance and repair become more complex when cleanrooms and secure rooms are involved. It is usually necessary for technicians to perform maintenance or repair work in these environments. This generally favors LIN units, since they require little maintenance. In addition, fewer tools are needed to repair a liquid nitrogen freezer, requiring less tool sterilization, cleaning, and bother. For example, one high-security government laboratory selected LIN freezers to reduce access by outside technicians.

Liquid nitrogen and CO_2 supply must be considered in cleanrooms and secure rooms as well. Any cylinders must be properly cleaned and validated before being brought in. One thing to consider is whether the supplier employs gas technicians with experience working with cleanrooms and secure rooms. A better option is to pipe in LIN or CO_2 from a nearby supply point outside the restricted room.

Managing the cryogen supply chain

Many cryogen users believe that efficiency of the supply chain and preservation of their samples are mutually exclusive. However, this is not only wrong, but it leads to needlessly wasteful decisions about the cryogen supplies. Streamlining the LIN and CO_2 supply chain can reduce costs, increase efficiency, and perform a better job of preserving samples. There are two issues to consider: 1) on-site inventory and 2) rental fees for cylinders. The costs for these can add up if not managed properly.

Implementing better cylinder supply procedures can result in incremental savings. End users are often willing to ignore costs if they can be assured of greater security for their samples. For this reason, they may keep excess cylinder supplies on hand. In the case of liquid cylinders, some of their backup supply will simply evaporate as it sits idle, or it may lead to practices that send usable product back to the supplier. For example, a laboratory will often replace the cylinders on its LIN units on a set schedule to help ensure an adequate supply, even though the containers have usable product. One large biotechnology laboratory in Boston, MA, changed liquid cylinders once a week to ensure cryogen supply, even though actual usage would have required a change once a month. The laboratory's justified obsession with preserving samples wasted 75% of its usable product, costing the laboratory $60,000 a year. Options such as automated changeover systems can achieve more complete withdrawal of product from the cylinder.

For example, many companies prematurely replace CO_2 cylinders that back up a mechanical freezer to ensure a fresh supply in the event of a failure. This can be expensive over time—a facility with 20 units might replace its cylinders once a month. At $30 a cylinder, this would cost over $7200 per year. However, by instituting simple measurement techniques, such as inexpensive scales to weigh the remainder, laboratories can track their supplies more effectively and reduce purchases.

Most laboratories make flat rental allocations in which each user is allocated a set portion each month. Since this expense is relatively low, many end users do not actively monitor cylinder balances; they simply want the LIN or CO_2 to be available when they need it. This leads to overstocking, with users keeping extra inventory on site even though their supplier may make several deliveries per week. This not only leads to excess supply but added rental costs as well.

When calculated across an entire laboratory complex, the added rental cost can be high. To help identify overstocking, one can calculate how long it takes a cylinder to travel through a site by dividing the total number of units purchased per month into the end-of-month balance. Many users are surprised to learn how long cylinders sit in their facility—often two for every one in use—adding unnecessary costs.

As previously mentioned, laboratories should consider the impact of the LIN supply mode on their operations. They may consider centralizing the supply through microbulk or

Gas and Cryogen Supply Options			
	Mode of Supply	SCF	Gallons
	Cylinders	10 to 350	N.A.
	Liquid Cylinders	4,500 – 5,600	48 – 61
	MicroBulk	5,000 – 48,000	61 – 539
	Bulk	> 45,000	> 500

Figure 4 *Gas supply options.*

bulk delivery. These larger supply modes can create cost savings by providing larger volumes at a lower cost (see *Figure 4*). They can also improve supply options to restricted rooms provided they are not too distant from the supply source.

This is where location plays an increasingly important role. In general, central supply is best for laboratories located on the ground floor, close to outside walls, and with adequate access for delivery vehicles. This is especially true for bulk or microbulk, because the further the source from the point of use, the greater the cost. Vacuum-jacketed piping costs approx. $150 per foot. Short runs are not expensive, but longer runs may add costs that offset other price reductions. In addition, many newer systems have built-in radio telemetry that enables the provider to monitor LIN levels and refill the tank automatically, reducing administrative costs.

Cylinder handling logistics also need to be factored in. It takes an average of 20 min to change a cylinder, depending on the location of the storage units. If nitrogen cylinders are delivered right next to the storage units, changeout time may only be a few minutes. However, if cylinders need to be changed on the upper floor of a building, the time can be much longer.

When considering the LIN supply chain, most facilities will maintain several supply modes. They may maintain a microbulk or bulk tank for their main supply and cylinders for backup and "spot" uses. Alternatively, they may want to have a second tank to shorten piping runs to harder-to-reach locations.

Proper safety precautions must be followed when working with both LIN and mechanical units. Due to extremely cold temperatures, cryogens will rapidly freeze human tissue. Therefore, insulated gloves approved for use in cryogenic service must be worn, especially when operating valves or when the potential exists for contact with product or exposed cold piping. Gowns, safety glasses, and face shields should also be worn to prevent injury to the face and eyes.

In addition, LIN produces large volumes of gas when it vaporizes. If a sufficient amount of liquid is vaporized, it can create health hazards, particularly if released in a confined space. For example, a small spill of LIN expands to 697 times its liquid volume when it vaporizes. This can displace oxygen, potentially creating an oxygen-poor environment. Therefore, LIN should be used and stored in well-ventilated areas. A reputable vendor should have the skills and experience to provide instruction in LIN safety and use.

Conclusion

Laboratories need to consider providing the best preservation of their research at the lowest cost and greatest convenience. At temperatures below –130 °C, LIN freezers in liquid or vapor phase will keep samples colder for longer periods of time and more efficiently. Yet, LIN freezers also compare favorably with –80 °C mechanical units in many applications. More than just the initial cost of the unit should be considered; all of the elements—temperature, sample integrity, power, space, location, maintenance, validation, security, supply, and safety—should be factored in when making a decision. The right choices become even more critical when the value of the research stored in the facility is considered. In addition, laboratories should view preservation of their samples and cost efficiency as equally obtainable goals. Through better tracking of cryogen supplies and reduced waste, it is possible to protect vital work while achieving immediate and long-term cost savings.

Finally, the laboratory should seek a supplier that is willing to work closely with its customers to understand its processes and recommend the solutions to meet its needs. Involving the supplier in the analysis will help to get the most out of gas and equipment purchases.

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