



Room Temperature Storage of Biological Samples

Room temperature storage of biological samples is an alternative to ultra low temperature frozen storage that can significantly reduce energy use while enhancing sample management and space utilization. This bulletin summarizes the technology and describes the costs, benefits and strategies for successful implementation, based on pilot implementations at Stanford and the University of California, Davis.

Introduction

Sample preservation in biology and medicine is essential for sophisticated biochemical studies of DNA, proteins, living cultures and viable tissues and hundreds of metabolites in all life forms. Freezer advances over the last fifty years provided ever-colder temperatures in mechanical freezers. Likewise the availability of liquid nitrogen storage has increased, allowing storage of cell lines and microbial cultures. Many of the samples in storage are irreplaceable, representing a career of research and providing an archive of samples for future assays and comparisons of disease, mutation, and metabolism in organisms long since deceased or extinct. While many samples are precious, a significant portion are obsolete or can be stored using less or zero energy, perhaps saving several hundreds of thousands of dollars per year.

Ultra low temperature (ULT) freezers are an essential storage device, especially for biological samples. The costs of each freezer are substantial and assumed automatically by host institutions. These ULT freezers consume about a kilowatt each, and reject about that much waste heat as well. Their inopportune breakdowns give them the reputation of “problem teenagers”. They are expensive to purchase, and are susceptible to malfunction in ways that are difficult to diagnose. While each may be “owned” individually by a principal investigator (PI), they are often the responsibility of a facility manager who has the burden to monitor, cool, back up and replace them when they fail.

Room temperature sample storage (RTSS) enables safe storage of biological material at room temperature. The technology prevents the degradation of biological materials at room temperature, eliminating the need for cold storage and cold shipping. Currently, biomolecules such as DNA, RNA and bacteria can be stabilized at ambient temperatures providing a cost-effective alternative to cold storage and shipping of samples.

Technology Description

RTSS is similar to extremophile biology [Crowe 1999] where organisms such as tardigrades and brine shrimp are able to protect their DNA, RNA, proteins, membranes and cellular systems for long-term survival in a dried state and later revive by simple rehydration (Figure 1). This technology mimics the natural molecular mechanisms used by these organisms. It creates a chemical lattice to support the molecules in their original shape without the dissolution properties of water. Both organic and inorganic solutes have been developed with equivalent lattice properties.

Additional molecules, whole organisms (living cultures) and even tissues may soon be addressed by this technology, but currently it is suited to only genetic material such as DNA and RNA over the long term. For short-term storage and shipping, some bacterial cultures may be preserved.

The method of storage is very simple. Most typical extracts of DNA or RNA may be pipetted into a small tube by hand, or a tiny well using robotics. The wells are either in 96-well or 384-well microtiter plates, which are standard in laboratories. They are pre-filled with a powder by the vendor, and the user opens and fills as many wells as needed. They then set the plate to dry either in a dust-free, laminar flow hood, or a chemical desiccator. The samples can remain in desiccators indefinitely without energy input.

These plates shrink the container size by about 90%, so the equivalent of an entire freezer full of samples may be transferred into a medium-sized moving box that sits on a counter or underneath it (Figure 2). This box does need to be sealed with desiccant, and high quality sealed boxes from a hardware store may be adequate for desiccated conditions

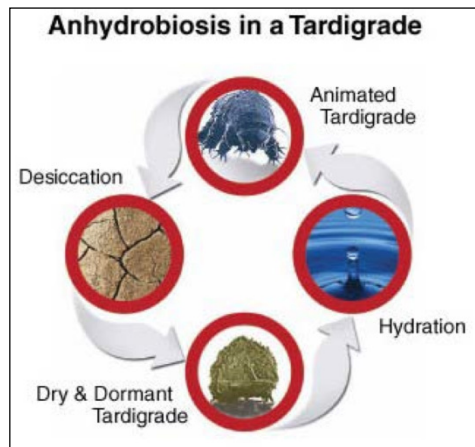


Figure 1. RTSS mimics organisms such as tardigrades, which are able to survive in a dried state (anhydrobiosis) and later revive by simple rehydration.



Figure 2. Two examples of benchtop room temperature sample storage cabinets.

A study by Wen et al. [2009] compared the performance of RTSS to frozen storage for a number of parameters. The study concluded that there was no loss of DNA or RNA quality stored at room temperature for 3 weeks and 11 days, respectively, as measured by short and long range polymerase chain reaction (PCR), DNA sequencing, DNA microarray analysis, and real time quantitative PCR. However, the study did not test longer storage, storage at upper and lower range, and did not evaluate multiple cycles.

Benefits and Costs of Room Temperature Sample Storage

The benefits of RTSS relative to ULT freezers include zero energy use, potentially reduced cooling systems, lower maintenance and reduced floor space. The scale and impact of savings is dependent on unplugging freezers or slowing growth of new freezers. Table 1 presents the operating costs for a typical ULT – which also represent the gross savings for each ULT that is unplugged and removed from a laboratory. (Amortized capital costs are not reduced by freezer retirement.)

Table 1. Annual operating cost estimates for ultra low temperature freezers.
These costs would be avoided with RTSS.

Cost Parameter	Annual Operating Cost		Assumptions
	Low	High	
Direct electricity	\$ 620	\$1,100	20-25 kWh/d, 8.5-12 cents/kWh
Heat rejection ¹	\$ 100	\$ 550	15-50% of direct electricity
Maintenance ²	\$ 200	\$ 400	\$50/h tech, parts for filters, tune, alarms
Floor space ³	\$ 400	\$1,200	20-30 SF, \$20-40 /SF/yr
Total	\$1,320	\$3,250	
<i>Amortized capital cost (not included)</i>	<i>\$ 500</i>	<i>\$1,750</i>	<i>Low: \$ 8,000, 16 years High: \$ 14,000, 8 years</i>

¹ While direct energy consumption is easily measured, cooling of reject heat may vary from 0-80% of input energy depending on freezer density, proximity to thermostats versus exhaust vents, recirculation vs. 100% outside air, climate, season and use of air economizers. For this analysis a narrower range was chosen in the absence of quantitative studies to formulate an average for hundreds of freezers.

² Maintenance costs include filter changes, alarm systems and occasional rebuilding of compressors.

³ Estimating floor space value is highly variable depending on space availability and accounting methods. Private sector costs may be substantially higher than academic institutions because floor space and amortized capital costs must be included.

There are additional benefits of RTSS not captured in Table 1. First, RTSS has a much reduced risk of failure due to mechanical breakdown, routine power outages, or natural disaster. These risks are difficult to monetize, but their value could easily exceed utility and capital costs when the contents of a single freezer may be worth hundreds of thousands of dollars. Second, RTSS does not require back-up electric power. Therefore, the cost of emergency generators in new construction or additional generators for existing facilities could be avoided or mitigated with extensive adoption of RTSS.

The costs of RTSS are varied, and provide energy managers with many leverage points to facilitate reduced mechanical freezing when subsidies are available. The cost to preserve one DNA extract may be as little as \$0.50 per well in a 96-well plate, or about \$1.50 in a microcentrifuge vial¹. These costs are reasonable when standard sample preparation expenses are included. For example, many purification kits cost \$20-100 for a batch of samples, and other disposable items may add up to \$2-3 per sample. The

¹ Products for RNA storage are substantially more expensive at this time, and universal protein storage media have not yet been developed.

energy and security payback comes a few weeks later when frozen samples are discarded because the dry sample is archived.

For an entire ULT freezer of already-frozen samples, RTSS costs are difficult to calculate due to the variety of samples and containers. Samples from DNA research are the most appropriate for complete inventory preservation and freezer retirement. A freezer may contain 5,000-15,000 vials, and the materials cost may thus be about \$2,500-8,000. Labor can be up to 100-200 hours, and may be performed by an undergraduate student hired by the researchers. Where robotic pipetting is available in a central facility, this cost may be reduced by sample addition to 384-well plates, which cost less than half of 96-well plates per sample, but are difficult for manual manipulation due to the small size of wells. The best opportunities for conversion also come at transition points for scientists - either when they first arrive on-site and a purchase may be avoided, or near retirement when inventory is pared down and precious samples are identified for long-term archival storage.

Passive storage cabinets are also needed to keep samples below 30% relative humidity, and may cost \$500-1000 per lab. These are typically rectangular airtight desiccators but less-expensive sealing plastic tubs may suffice in dry climates. Plexiglas cabinets could also be made on-site with standard woodworking materials, adhesives or solvents, and hardware.

For typical labs with proteins, tissues and cultures, it may be more appropriate to convert one shelf of samples, which will delay or eliminate purchasing the next freezer. For labs that have four or more freezers, sample consolidation may make it possible to empty and retire an entire ULT freezer.

Freezer Growth – Case Study

The growth of ULT freezers and future scenarios at UC Davis are shown in Figure 3. Freezers have approximately doubled in the last ten years, resulting in over 800 ULT freezers on the Davis and Sacramento campuses. While frozen sample growth is projected at 10-15%, conservative business-as-usual trends of 1) 8% growth and 2) arithmetic growth of 65 per year are graphed here (the latter value represents recent annual purchases at UC Davis). Given the likelihood of energy price escalation, aggressive greenhouse gas reduction policies and the risks of mechanical preservation these growth curves are untenable.

Two scenarios for future ULT storage were chosen: 20% and 40% diversion. The lower value is based on an estimate of samples in storage at Stanford University that are appropriate for RTSS. The higher value includes aggressive consolidation of obsolete samples as well as expanded preservation capacity of proteins in the future. The savings are about 700kW of direct electric power in 2020 (excluding cooling savings). Consolidation includes disciplined sample management using inventory software as well as RTSS transfer incentivized by freezer fees and rebates. This does not include attrition of current inventory which would add to savings and is an essential management target.

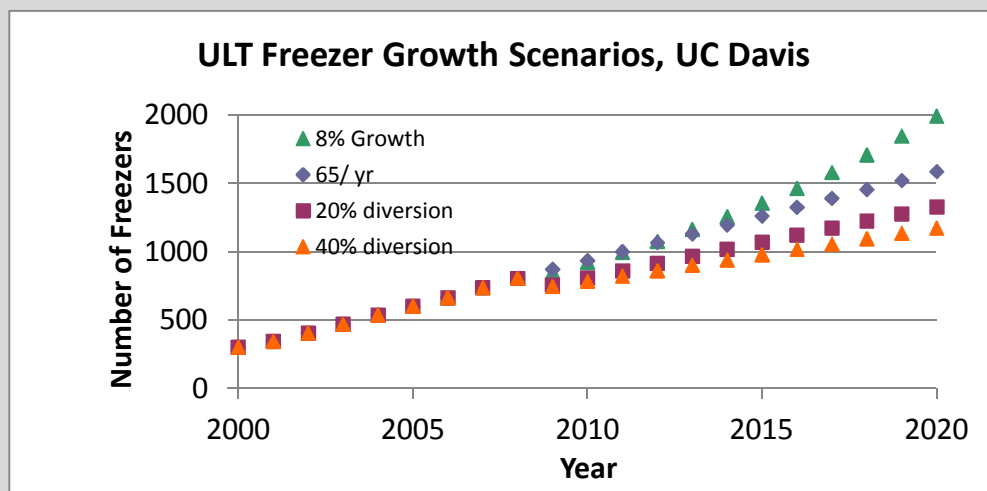


Figure 3. Growth scenarios of ULT freezers at UC Davis.

Overcoming Barriers to Adoption

Scientists are skeptical and even dismissive of methods like RTSS despite the verification by their peers, such as the study by Wan et al. [2009]. Thus energy managers and sustainability officers need to host technical demonstrations, webinars, or vendor displays to develop confidence in the technology. It may also be helpful to provide free RTSS materials on a trial basis to win over a few key researchers. Once prominent colleagues have adopted RTSS, others are more likely to try it in their own labs.

For archival purposes the best time to store samples dry is immediately after active sample experimentation, which diverts them from deep freezing. For new projects this opportunity needs to be incentivized and promoted to be an attractive option and standard procedure. Dried samples may be re-hydrated and re-dried several times with excellent recovery, although it is not as convenient as freezing and thawing. On the other hand, an entire plate of samples may be removed from storage and a single sample re-hydrated without risk to adjacent samples.

Converting existing archives of samples to dry storage presents a significant challenge when one freezer may contain 5,000-15,000 samples [Jensen 2009]. The time required to convert, materials cost and organizational inertia inhibit optimized sample management. Campus energy managers can motivate researchers with cash, labor (human or automated), or materials subsidies. For instance, paying an undergraduate student for manual transfers is an excellent gateway experience to molecular biology. Central laboratories or “core facilities” often have robotic pipetting equipment that can greatly facilitate this transfer at minimal cost compared to manual transfer.

Another incentive that energy managers may use is providing sample management software (SMS) as part of joining the cold storage initiative at a reduced price. Sample labeling, organization and management is crucial to research, and this may be an attractive way to highlight valuable samples as well as put expiration dates on others. Software selection requires a good partnership between energy managers and research support, and can empower sample access while facilitating energy conservation.

RTSS materials may be subsidized on storeroom shelves or through rebates. Early adopters should obtain the best subsidies, while eventually subsidies may be reduced so that researchers do not acquire excessive dry storage supplies. With each subsidy an inventory of all samples can be very informative for future storage and to get measures of success. Cash subsidies can be offered for a variety of steps, from cleaning out old samples to free up space, or converting one shelf of a freezer to dry storage, to completely emptying a freezer and relinquishing it for recycling at an approved plant. Such cash rebates have been offered at Stanford University and are under development at UC Davis. Researchers consulted at UC Davis agree that eventually an annual fee may be necessary to discourage freezer use. A fee rebate can be offered with demonstration of good sample logging, freezer defrosting and filter cleaning.

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