-70 is the new -80

Lab managers from around the world explain how they cut energy costs, lowered their carbon footprints and prolonged the life of their ultra-low temperature freezers. The secret? Clean out freezers and make sure energy-efficient freezers are well maintained. In addition, raise the temperature of the ULT freezers from -80^{o} to -70^{o}.

Radboudumc

The temperature setting of freezers

Ultra-low temperature (ULT) freezers are among the most energy-intensive pieces of equipment in laboratories. When set at -80°C, ULT-freezers may use up to 20kWh per day. This is as much as entire households [1] and accounts for >900 US$ in electricity costs per annum.

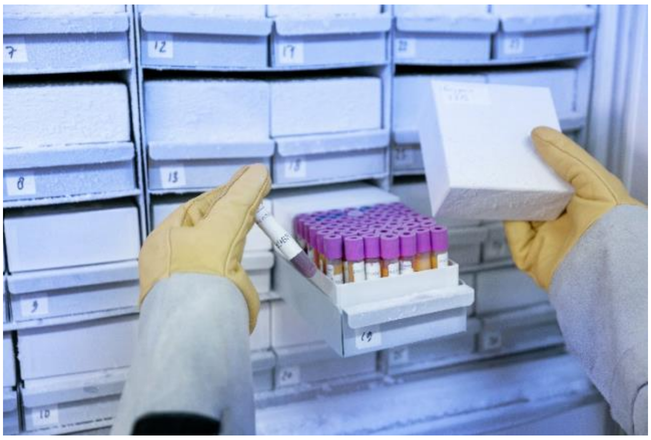
The exact energy use is influenced by the freezer-model (old ULT-freezers can use more than threefold more energy than newer models), the time the freezer has been in use, freezer capacity, ambient temperature, ice, dust and the way freezer content is organized (i.e. how full the freezer is and spacing between boxes) [1,2] .

Importantly, ULT-freezers used to be set at -65°C or -70°C [3]. Only in the 1980s-1990s did producers of ULT-freezers start to advertise lower temperatures of -80°C or even -86°C. Although this 10°C lower temperature comes at a considerable cost (up to 30% higher energy use [2,4]), no evidence was provided that lower temperatures improved sample stability or recovery. The crystallization (freezing) point of water (0°C), the 1st re-crystallization (-60 to -63°C) and 2nd re-crystallization point (-130 to -135°C) are critical temperatures for long-term storage of samples; -80°C, however, is not a critical temperature.

In recent years, many labs have reverted to the original ULT freezer temperature to meet sustainability objectives, prolong the life of freezers and save money [5]. Also producers of ULT-freezers acknowledge this new reality. [3]

Freeze-thaws

Although -70°C was the standard ULT temperature for many decades, it may now feel uncomfortable to increase the temperature of freezers holding precious materials. One concern could be that freezers may become more prone to freeze-thaw in case of power-cuts. A study that was designed to address this concern reported that keeping a freezer at -80°C will buy you only 35 additional minutes before reaching -20°C in case of a complete power failure compared to a freezer set at -70°C (19 hours 10 minutes compared to 19h 45m) [6]. For a well-maintained freezer with fully closed door, there is 6% less time to save your samples if your freezer is set at -70°C and one would still have >36 hours before thawing [6]. Obviously these scenarios should be hypothetical for freezers that are on a backup circuit or are equipped with an automatic alarm signal. The temperature impact of opening doors is similar between freezers set at -80°C or -70°C [6]. Freezer content organization, however, is critical for temperature stability in case of prolonged opening or power failure: a partially empty freezer heats up considerably faster than one at capacity.



Sample integrity

Direct comparisons of sample stability/recovery when stored in -70°C or -80°C ULT-freezers are rare. Whilst no such comparisons were needed to market energy intensive -80 or -86°C freezers, there is an understandable desire to ensure sample integrity when a switch in the opposite direction is considered. For some sample types there is data about stability in different storage conditions. Genomic DNA is stable at -20°C or -70°C [7]; similar stability and viability of fungal isolates was achieved after 8-year storage at -70°C and -130°C[8]. Similarly, no differences were observed in a series of assessments of serum antioxidant status when samples were stored for 1 year at -20°C, -70°C or -196°C [9]. Plasma antibodies against HIV, HCV and HbsAg were stable for over 15 years at -20°C [10], and cardiac troponin T plasma concentrations are stable for over 8 years when stored at -70°C [11].

We made the switch to -70°C

Given that not all sample types have been extensively tested and there are direct few comparisons of -70 versus -80°C, some representatives of prominent labs who made the switch to -70°C shared their experiences.

John Grist is senior lab manager at the Wolfson Center for Age Related Diseases at King's College London in the UK. He is responsible for a lab with 18 ULT-freezers that are used to store DNA, RNA, protein samples, animal and human tissue. All 18 freezers are currently set at -70°C. For him, the decision was easy.

'I couldn't find any scientific reason why manufacturers decided to produce freezers with -80/ -85°C as default temperature. Some years ago I gave researchers in my group a chance to produce evidence that -80/85°C storage was better than -70°C. Nobody came back to me so I changed the temperature of all 18 ULT-freezers to -70°C. We see no indications of a loss of sample integrity.'

Kerra Pearce is senior lab manager at the Institute of Child Health at University College London (UCL) in the UK. Together with 4 colleagues, she is responsible for the lab infrastructure for roughly 100 Principal Investigators (with 700 staff members over 90 lab spaces). This large team makes use of 67 ULT freezers for a variety of samples, including RNA, tissue and cell lines.

Starting in 2017, temperatures were changed from -80°C to -70°C. At present 46 of 67 ULT-freezers are set to -70°C. 'There was initially some pushback from investigators and concerns about sample integrity. In the end it was a combination of PIs supporting the initiative and a top-down decision of the Director that led to nearly two-thirds of our ULT-freezers being set to -70°C. The few ULT-freezers that are still at -80°C are 10 freezers from PIs who are currently reluctant to make the step and those containing samples for quality assurance schemes or stored under the Human Tissue Act'.

Allison Hunter is Technical Operations Manager at the Department of Life Sciences, Imperial College London in the UK and prior to that was a technical resource manager at King's College London (KCL) for highly serviced life sciences research space across two campuses for 1600 staff. She started the first lab freezer sustainability project at KCL in her labs in 2008. Her approach was to ask for a reduction to -70°C on a voluntary basis after demonstrating to the researchers that this would save 260 US$ and 1 ton of CO_2 per freezer per annum on average. Initially 19 out of 63 -80°C freezers in her labs were changed to -70°C within a 3 week timeframe, and she facilitated agreement between different groups sharing the same freezer.

Freezers set at -70°C contain samples including plasmid glycerol stocks, OCT embedded blocks for cryosections (mostly brains of mouse, rat and chicks), bacterial competent cells, *Drosophila* tissue or whole flies flash frozen in liquid nitrogen, antibodies and pharmaceutical and nutrition research samples. The program, including voltage stabilization, continued to roll out across the College after she moved to Imperial.

The full audit of the university, supported by a HEFCE Catalyst Grant awarded to her and Kat Thorne and matched by College funds, showed that 2000 freezers cost about £450,000 (592,000US$) per annum for power alone. There were 215 ULT freezers in total. Cold storage is a substantial energy and footprint cost for universities.

This work was recognized by the first King's College London Sustainability Award in 2014 and a S-Lab Making a Difference Award in 2015. She has also lobbied the European Commission to have greater attention to lab freezer manufacturing standards recognized by EU directives.

Kathryn Ramirez-Aguilar has managed the CU Green Labs Program at University of Colorado Boulder (CU Boulder) in the US since 2009 after completing a post-doctorate in biochemistry. The program focuses on engaging scientists and working in partnership with campus stakeholders to optimize energy efficiency in scientific laboratories.

'In 2010, when the idea of -70°C had been suggested to me by a campus scientist, I discovered ~5 ULT freezers on campus that had always been set at -70°C. Over time, especially through CU Boulder's participation in the Freezer Challenge, more and more CU Boulder laboratories have voluntarily chosen to set their ULT freezers at -70°C.

Now about half of the ~175 ULT freezers at CU Boulder are set to -70°C. These ULT freezers are used by scientists in many fields of study including molecular biology, integrative physiology, engineering, geology, biochemistry, and ecology. Additionally, CU Green Labs began an effort in 2016 to provide campus researchers with shared ULT freezers where scientists pay a small fee to rent space.

We have 4 energy efficient ULT freezers set at -70°C serving about 80 researchers from 20 different research groups with a wide range of different sample types. It has been inspiring to me to see so many CU Boulder scientists choose to store their samples at -70°C. Some labs even changed the temperature of their freezers without letting CU Green Labs know. I discovered they had made the switch years later.'

Jessica Henley has been the lab manager of the Noah Fierer Lab since 2012 in Cooperative Institute for Research in Environmental Sciences at the University of Colorado Boulder in the US. The Fierer lab studies environmental microbiology with a focus on soil microbiome communities. 'We haven't noticed any change since switching our ultra-low temperature freezer from -80°C to -70°C. We have been storing reagents and culture stocks at -70°C since 2013 and have had no problems. Happy to be lowering our energy usage without changing the quality of our work!'

Rachel Tapp of Charles River Laboratories is a senior research scientist and the chair for the sustainability champion committee at the Mattawan (Michigan, US). 'As our site primarily performs studies under the Good Laboratory Practices (GLPs), it was important to completely understand and follow appropriate guidelines for our sample storage units that meet these rigorous demands.

Initially, each ULT system we receive is temperature mapped to ensure quality storage. In addition, all systems are connected to our temperature monitoring systems which are monitored and attended to 24 hours a day, 7 days a week.

During our evaluation of our freezer systems, it was determined that a range of -60°C to -90°C is considered equivalent and was set as our standard acceptable range for the use of ultra-low freezing units. Our site also developed appropriate standard operating procedures to govern these systems and set the ULT systems accordingly.

All our systems are also barcode mapped, so samples can be quickly accessed and stored with minimal "open door" time. All ULT systems are maintained on schedule by our metrology and facilities departments to ensure they are properly working over their lifespan. A well-maintained ULT, set to meet the ranges of our standard operating procedures, meets the demands of a GLP environment in our laboratory.'

Mark de Graaf, research technician, and **Johan van der Vlag**, Professor and head of the Nephrology research laboratory, both work at the department of Nephrology at Radboudumc, the Netherlands. Their department has 9 ULT-freezers in use to store laboratory research samples, such as cell and tissue lysates, purified proteins and DNA/RNA, as well as plasma and urines of patients with kidney disease. As part of an institutional "freezer challenge", they discarded 119 boxes of samples that had not been touched for many years, and changed the temperature setting of all their ULT-freezers from -86°C or -80°C to -70°C. 'We tried to find scientific evidence that could justify a temperature of -80/-86°C over a temperature of -70°C to store our samples. However, since we could not find any evidence, we increased temperature of all our freezers to -70°C. Eight old, out of date and economically depreciated ULT-freezers were retired and replaced by latest energy saving models. 'We measured the energy consumption of our freezers and found that freezers bought in 2007 used twice as much electricity compared to those from 2015. This difference was quit confronting, thereby realizing we had even older freezers in use…. Setting the temperature to -70°C resulted in an additional 26% reduction in energy consumption. We joined the "freezer challenge" because we as a department of Nephrology highly value sustainable research and patient care, in line with the mission of Radboudumc. We should aim to reduce the ecological footprint from our activities to an absolute minimum, that is energy neutral at the end, whereas recycling of waste products should be maximized'.

Arjo Meijering is an engineer responsible for energy, safety and sustainability at Wageningen University & Research (WUR) in The Netherlands. With colleagues, he has developed a new approach to ULT sample storage at his university. For samples that are not used on a weekly basis, central freezing facilities are provided. The system that is in place in Wageningen saves up to 70% energy compared to storage in individual ULT freezers set to -80°C.

The most recent ULT central storage facility (45 m^3 with place for 2.000.000 eppendorf tubes) is set to -70°C to save energy. Whilst he acknowledges that there are no studies that directly compare sample integrity between storage at -70°C versus -80°C, he indicates that these temperatures can be misleading. 'In fact there can be considerable variation in temperature within older ULT freezers.

Some ULT-freezers fail to achieve this temperature for most of the storage space. Unknowingly, many researchers have been storing their samples at temperatures that are considerably higher than -80°C and achieved good results. -70°C is perfectly acceptable for most samples, though there are exceptions (we still store our RNA samples at -80°C). A well-maintained large freezing facility at -70°C is probably as good or even better than individual ULT-freezers set at -80°C, because of a more stable temperature.

John Verbeek is operational manager at the department of medical microbiology at Radboudumc, the Netherlands. His department had 19 ULT-freezers that are used to store microorganisms (bacteria, parasites, viruses, fungi , yeast and patient materials). Somewhere in 2019, he started a rigorous cleanout process of old samples and discarded over 500 boxes of samples. In addition, he changed the temperature setting of 17 ULT-freezers from -80°C to -70°C and retired two very old freezer models. This resulted in considerable savings in terms of electricity costs and carbon emissions. Moreover, there are now very energy-efficient systems on the market, that can replace stand-alone ULT-freezers. In recent years John and colleagues drew up a plan to build a central freezing facility at Radboudumc. It is expected that at the end of 2021 a start will be made with installing the system, with a capacity of 60,000 liters, that can be expanded to 130,000 liters if necessary. This will result in enormous energy savings for all materials that are not needed on a monthly basis. 'For remaining stand-alone freezers at departments, -70°C should be the default temperature'.

Sabrina Jenull is postdoctoral researcher in the Kuchler lab at the Max Perutz Labs in Vienna. Her institute harbours 65 ULT freezers that all used to operate at -80°C. Early 2020 one floor (out of five) uniformly raised the temperature of all 6 ULT freezers at this floor to -70°C. This action was decided upon after a democratic vote among the PIs and technicians during their monthly meeting. An additional group switched one of their ULT freezers from -80°C to -70°C and started to offer a space for other groups to test sample storage at -70°C instead of -80°C.

'So far, we did not have any issues in terms of the stability of our samples, which include fungal and bacterial strains and cell lysates used for RNA and protein extraction. There were no complaints from the research groups about the switch. At our campus we are not the only institute taking on the freezer challenge. Other institutes raised the temperature of their ULT freezers about a year ago in combination with exchanging old freezers with energy-efficient ones.'

Author and references

This document was written and compiled by Teun Bousema (Radboud university medical center, Nijmegen, The Netherlands) for the Radboud Green Office, in collaboration with Allison Hunter (Imperial College, UK), Kathryn Ramirez-Aguilar (University of Colorado, US), Martin Farley (LEAF – UCL, King's College London labs), Jeroen Dobbelaere (Climate@MaxPerutzLabs, Vienna, Austria) and Christina Greever (mygreenlab.org). The current version is dated 1st of September 2020; regular updates are anticipated.

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