Long Term Sample Storage at the LifeLines Project

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In Biobanking, sample security is of paramount importance. Thousands of potentially irreplaceable samples may be kept over several years for use in research, and it is vital to know that storage systems accurately maintain the required temperature and are reliable enough to keep them safe. At the LifeLines project in the Dutch province of Groningen, this need is keenly understood and the team has selected its storage systems with great care.

The LifeLines project

The LifeLines project was set up in 2006 with the aim of becoming a large scale genetic study lasting for 30 years and including around 165,000 participants spanning three generations, each providing researchers with biologic samples for analysis. The project's main aim is to investigate why some people remain healthy and active throughout their lives while others become disabled at a relatively early age. Particular attention is being paid to chronic illnesses such as diabetes, kidney failure and asthma. In order to develop a clear understanding of how and why these develop, the LifeLines project examines a range of factors that contribute to a person's general health and risk of developing chronic diseases. These include lifestyle, genetic, psychological and social factors.

Results and information gathered during the LifeLines project is expected to enable the development of individualised prevention and treatment programs. Chronic illnesses tend to follow a pathway, and if they can be detected and defined at an earlier stage there is the potential to save the healthcare system time and money, as well as to dramatically increase the comfort of the patient.

One example of such an application is the treatment of diabetes, which is becoming increasingly prevalent and impacting healthcare economics. In patients where lifestyle adjustments can be made, having information available early can help healthcare professionals make informed recommendations for change. Being able to track and relate to the information provided can be a real motivator for patients to make modifications that may help slow disease progression.

The research conducted at the LifeLines project has already been making an impact, having been included in several publications. These include 'Automated mass spectrometric analysis of urinary free catecholamines using on-line solid phase extraction' by De Jong et al (Bio med Life Sci 2010; 878(10); 1506-12), where reference values of urinary excretion of adrenaline and noradrenaline were obtained from more than 500 LifeLines participants.

Figure 1. Multiple Panasonic, formally Sanyo, ultra-low freezers in use at Lifelines Project

Selection of sample storage equipment for the LifeLines project

Overall, the LifeLines project expects to hold a total of 8 million 1.4 mL 2D-coded sample tubes (Matrix and FluidX). Depending on the health of the participant, 50% of these samples may be held for the entire duration of the project, and so reliability of the storage equipment is crucial.

Initially, up to 100 freezers were needed before the samples will move to the Bios, the robotic -80 freezer, and all equipment had to meet strictly defined standards. Temperature stability was particularly important to the researcher, as experience had shown that some freezer models exhibited temperature gradients from the top to the bottom of the freezer, or heat leakage from around the door.

During the selection process, products from four freezer manufacturers were tested to see how well they met the specifications. The evaluations included temperature tests on 3 to 4 different spots inside each freezer compartment, based on a specified temperature of approximately -81°C with a maximum accepted temperature deviation of -7°C between the coldest and warmest spot within the internal chamber.

Freezers then underwent stress tests, where the doors were opened for 1 minute, closed again for 30 seconds and then checked to see how well they held and recovered their programmed temperatures. As the freezers were likely to be opened and closed on a regular basis to access the samples, it was crucial they didn't warm up too much or take too long to stabilise.

Finally, power consumption was monitored over 7 days to ensure energy efficiency.

After testing was complete, Panasonic ultra-low temperature (ULT) freezers were selected for the Lifelines project after demonstrating their ability to easily achieve the required standards in price, quality and temperature stability.

Cost efficiency with long-term storage solutions

The LifeLines project is not alone in considering running costs and energy consumption as part of purchasing decisions. These are fast becoming leading factors across many laboratories, especially as the long-term storage of large numbers of biological samples becomes more common.

Panasonic was the first company to introduce vacuum insulation panels in its ULT freezers. The range typically provides 25% more storage capacity for a given floor area, saving valuable laboratory space and running costs in institutions such as the Lifelines project, where multiple freezers are required.

The option of a water cooled condenser within the cascade cooling cycle of the selected freezers means that heat removed by the condenser can be conducted across a heat exchanger and channelled out of the system through exiting water. This translates into less heat generation by the instruments themselves, allowing laboratories to make dramatic savings on air conditioning systems.

Furthermore, heat extracted in this way may be reused elsewhere in the building, for space or water heating, for example.

Freezer technologies ensuring sample security

Freezer failure or temperature fluctuations could result in the loss of samples, with dramatic implications for the study, both financially and in terms of the research being compromised. A range of technologies and systems go a long way to ensuring that the researchers at the LifeLines project can continue with their work knowing that the samples are protected.

Advanced microprocessor controls in the freezers selected for the project constantly monitor function, including system and ambient conditions, for reliable operation. With comprehensive setpoint, alarm, monitoring and diagnostic functions continuously taking place, every aspect of the freezer environment is checked on a regular basis to ensure samples remain secure. The system alerts users to any abnormalities before problems set in.

If a fault should ever occur, extensive audio-visual alarms and remote alarm contacts ensure that Lifelines staff are alerted immediately. High temperature warning equipment indicates when the temperature inside the freezers deviates ±10°C from the set temperature. A power failure alarm lamp and buzzer will activate in the event of power outage or irregular temperature increase. An additional alarm and temperature monitoring system was also able to be installed at the LifeLines facility, giving a second layer of security to the stored samples.

Figure 2. Multiple Panasonic, formally Sanyo, ultra-low freezers in use at Lifelines Project

Since the purchase of the freezer system by the LifeLines project, an updated model has been produced by Panasonic. This latest model incorporates a Twin Guard Dual Cooling system, specifically designed to provide the highest possible security. Having two individually controlled compressors not only maintains an ultra-low temperature within the freezer chamber, but also means that should there be a problem with one compressor, the second will maintain a working freezer temperature of -70°C until service is arranged.

Valuable contributions to biobanking

The success of the investigations carried out in Biobanking facilities depends on the samples being maintained in optimum condition – something which is highly dependent on the storage techniques and equipment. At the LifeLines project, the Panasonic freezer system is used for preserving the samples from thousands of participants while still maintaining the units' lowest possible energy consumption. To ensure viability, each and every sample is kept in a stable, reliable environment, and is constantly monitored to ensure that optimum temperatures are maintained over many years.

Thanks to having such a large and well-maintained collection of sample information, the LifeLines project is already contributing to a range of well-received studies. The project looks set to go from strength to strength, and will further our knowledge of healthy aging and chronic disease.

Figure 3. LifeLines project storage facility

Keep Paraffin Blocks as Cold as Ice

Histological examination is considered the gold standard for detection, diagnosis and characterisation of many clinical conditions, and producing suitable sections for microscopic examination constitutes one of the primary skills of the histologist.

Preparing and embedding tissue in a suitable matrix enables the technician to produce sections of an appropriate thickness for pathological analysis. Paraffin wax continues to be the most popular embedding medium, due to its low cost and ease of use, facilitating long-term storage of the embedded tissue blocks. To promote good ribbon generation, the paraffin block needs to be sufficiently hard to enable the sections to be cut on the microtome.

This is routinely achieved by placing the paraffin blocks on ice, cooling both the tissue and the wax to a similar consistency and swelling the tissue to make it easier to section. As the block heats up, however, the paraffin softens and expands, resulting in corrugation and variations in the consistency of the sections, rendering some unsuitable for use.

Technicians therefore have a finite time in which they can section and, as a result, have to continually remove the block from the microtome and re-cool it on ice to achieve the quality of sections required for downstream analysis, impacting workflow and laboratory throughput.

The **Thermo Scientific** Cool Cut Clamp is a peltier-cooled specimen clamp which helps to address this issue by maintaining the paraffin block at a suitable temperature for sectioning for a prolonged period. Compatible with all the Thermo Scientific HM range of rotary microtomes, the Thermo Scientific Cool Cut provides laboratories with the ability to achieve consistent section thickness when cutting individual, step and serial sections, with minimal interruptions.

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