Challenges of a biobank regarding cryogenics

Experience of an university medical center-integrated biobank

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Abstract

Over the last ten years, the awareness of the problem of partially irreproducible research results has increased. Many approaches have been discussed and implemented to overcome this problem. In biomedical research, an important factor for the irreproducibility of results is improper handling of biospecimens. Biospecimens are subject to degrading processes that are inhibited by storing the biospecimens at low temperatures. This is why maintaining a strict cold chain is crucial.

Establishing central biobanks at university medical centers is a key approach not only to overcome the challenges of irreproducible research results in biomedical research but also to support researchers in biospecimen logistics with standardized processes. Even though biobanks have implemented standardized processes and have specifically trained personal, their main challenge is to maintain the cold chain at any time once the samples are frozen.

In this work, three categories of cryogenic challenges encountered by biobanks to maintain the cold chains of frozen samples are described. For most of the challenges, at least conceptual ideas for solution approaches exist, in some cases even prototypes. A common theme between these challenges and solution approaches is a need for better insulation of mechanical parts to withstand ultra-low temperatures. To get fit-for-purpose solutions for their everyday challenges, it is critical that biobankers work closely together with (cryo)engineers to collaboratively develop solutions.

Key words:

Biobank, challenges, cold chain, insulation

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1. Introduction

2. Material and Methods & Results

In the following, we present three different categories of challenges that biobanks are facing in their daily operations: (1) attaining accurate temperature measurements of biospecimens, (2) enhancing non-automated storage devices, and (3) handling of the samples while maintaining ultra-low temperatures. For each of the problems, the availability of solutions (not available yet, existing idea, available prototype) is indicated as a level of maturity of the solution.

2.1. Accurate temperature measurements

Throughout steps 4 to 7 in Figure 1 i.e. while the biospecimens are handled by the biobank, the gold standard is to measure the environmental temperature surrounding the biospecimens continuously. However, because of multiple reasons this might be to imprecise to accurately assess the temperature of the biospecimens themselves within the storage tubes. A wide array of temperature loggers is already available, but with the large dimensions and the moving parts of automated storage systems, common wired temperature loggers seem impractical. See Table 1 for an overview of challenges regarding accurate temperature measurements. These challenges will be explained in more detail in the following sections.

Table 1: Summary of selected challenges in the category of accurate temperature measurements

Challenge	Possible Solutions	Maturity of solution
Temperature gradient in large storage areas	Develop an appropriate mobile temperature logger to assess temperature gradients accurately.	Prototype
	Develop ventilation systems to distribute the cool air evenly.	Idea
Temperature changes in biospecimens due to transfer between different environments	Develop a mobile temperature logger, that withstands ultra-low temperatures (-80 °C and -196 °C) for extended periods, to assess temperature changes in biospecimens accurately.	Prototype
	Develop insulation for transfer devices inside automated storage systems or insulation for SBS racks.	Idea
Calibration	Necessity of calibration should be considered early in future iterations of storage robots, to provide access to the positions of temperature sensors.	Idea

Temperature gradients

Looking into step 5 of Figure 1, while biospecimens are stored in biobanks at ultra-low temperatures, the temperature inside all storage devices is monitored continuously. However, since the storage devices are sometimes quite large, measuring the temperature even at a couple of positions seems to be too imprecise. The storage robot at the Central Biobank UMG for instance has two storage areas with a size of roughly 9 m^3 (5,06 m x 2,48 m x 0,73 m) per unit (see **Figure *2***).

Instead of measuring the temperature at few fixed points in the storage devices, measuring the temperature of biospecimens within the storage tubes while stored in different areas of the storage devices [27] gives an indication on the effective and relevant temperature differences between different parts of a storage area. However, the battery life at cold temperatures keeps being a limitation.

Better insulation for these temperature-logging devices is a topic for future work to prolong the single measurement intervals. Once, this is solved, the next step would be an approach using several mobile temperature loggers simultaneously.

Alternatively, future work can try to develop refrigeration systems where the temperature is more homogenous throughout cooling devices so that few fixed measurement points give an accurate estimate of the temperature at all positions. A ventilation system might help distributing the refrigerated air more evenly.

Temperature changes in biospecimens due to transfer between different environments

Furthermore, in steps 4 and 7 in Figure 1, the biospecimens are transferred between different locations. In automated storage devices, biospecimens are also moved between different areas within the device, which can have different temperature set points. For example, the storage robot at the Central Biobank UMG has two storage areas with temperatures of -80°C and a scanner module and aisles at -20°C. This further complicates the continuous temperature assessment and affects the quality of different types of biospecimens in the long run.

Measuring the temperature of biospecimens within the storage tubes while moving biospecimen between different devices or areas within devices [27] gives a realistic assessment of the temperature of the biospecimens. This in turn gives a good indication of which critical steps in the biobanking process endanger the cold chain.

As discussed above, the battery life at ultra-low temperatures is a limitation on any temperature logger. This is especially the case for temperature loggers at -196 °C. A logger that could withstand temperatures that low for an extended time would be very valuable in also identifying crucial process steps for biospecimens stored at -196 °C. After temperature sequences are acquired this way, the effects on biospecimen quality need to be determined.

Ideas to tackle these problems include developing insulation for the transfer devices inside automated storage systems, such that the exposure of racks with biospecimen to warmer temperatures is mitigated, and developing SBS racks with integrated insulation to minimize the exposure through all process steps.

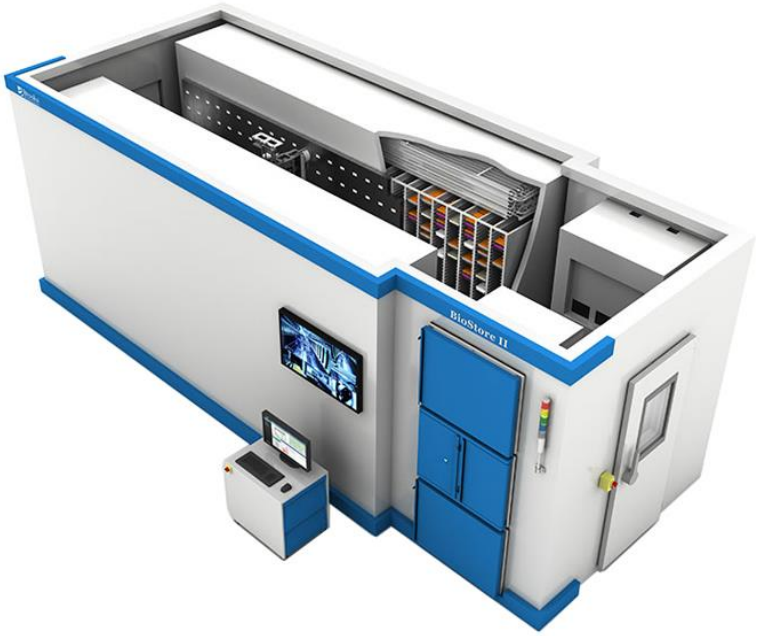

Figure 2: Simulated view of the Azenta BioStore II™ at the Central Biobank UMG. The black tiles cover and thereby border the long-time storage area. The orange parts inside this storage area depict shelves of biospecimen containers. On the other side of the aisle is another mirrored storage area of the same size. (Image courtesy of Azenta Life Sciences, Manchester, United Kingdom).

Calibration

Another challenge is the calibration of permanently installed temperature probes. These probes are usually placed at positions inside the storage devices that are not easily accessible, e.g. at the back of the interior wall or in between cooling parts (see **Figure *2***). Installing a ductwork for cables to insert calibration sensors does not seem sensible because this would create a cold bridge for these critical temperature areas. Nevertheless, working with calibrated thermocouples is critical for biobanks.

2.2. Enhancing non-automated storage devices

When furnishing a biobank initially, it is very difficult and complex to estimate the requirements towards the cooling devices. There are multiple different solutions including different devices with different storage temperatures (-4 °C, -20 °C, -80 °C, -196 °C). Apart from the total amount of storage capacity needed in relation to different temperatures, an initial planning also needs to take into account the necessary throughput, i.e. how many samples can be stored or retrieved in a given time.

There are many different types of highly efficient automated storage equipment available on the market [21]. However, these are oftentimes quite expensive. For the same total capacity, the price in acquisition for automated storage devices can be expected to be at least five times higher compared to non-automated devices to our experience.

Apart from the high-throughput, automated systems have further advantages:

- Single biospecimens are not exposed to room temperature while being condensed on racks or picked for retrieval.
- The handling of biospecimens is standardized and every biospecimen is treated the same.
- All processes are meticulously documented.
- The processes need less man power with less manual handling.
- There is less ice-buildup on the biospecimens as there is less humid air inside automated systems.
- Automated systems usually have a more redundant cooling setup.

But even if the requirements suggest an automated system and the necessary funds are available, the size of potential storage devices can also be a deciding factor. The requirements of automated systems with regard to ceiling height and floor load are generally higher. This makes them often infeasible for small rooms or rooms above the ground floor.

Developing enhancement products for existing non-automated storage devices could close the gap between the sometimes unnecessarily expensive, large automated systems and the smaller, less efficient non-automated versions and help biobanks find appropriate storage solutions.

Products like this would also help in case initial requirements need to be adjusted. Since most biobanks are just being established, changing surrounding conditions like additional funding and a drastic rise in usage need to be met with appropriate hardware solutions without the massive investment and ecological dilemma of replacing entire sets of storage devices. To support specific tasks in regular Biobanking processes, we propose the development of additional hardware for automation to overcome the challenges described in Table 2.

Table 2: Summary of selected challenges in the category of enhancing non-automated storage devices

Challenge	Possible Solutions	Maturity of solution
Lifting storage towers from liquid nitrogen tanks	Develop an aid that can operate at ultra-low temperatures to lift up towers.	Idea
Condensing biospecimen tubes on SBS racks	Building upon existing automated systems, develop a standalone device for automated picking/condensing of biospecimen containers.	Idea
Picking biospecimen tubes for selected retrieval	Develop a semi-automated mechanical device that lifts biospecimen containers upon request from a LIMS.	Prototype

Lifting storage towers from liquid nitrogen tanks

One of these tasks is for example the lifting of storage towers from liquid nitrogen tanks. With non-automated tanks, the storage towers need to be lifted manually to retrieve single boxes from storage (see Figure 3). One

tower filled with samples can weigh up to 4 kg. If samples are stored or retrieved from the lower-most shelf and the tower needs to be lifted all the way up, this can be challenging and physically fatiguing.

Figure 3: Manually retrieving a storage tower from a liquid nitrogen tank (copyright Central Biobank UMG).

A mechanical aid that helps pull up a tower while withstanding the ultra-low temperatures would be a great way to enhance the handling of non-automated liquid nitrogen tanks. In addition to the practical support of the handling of towers in liquid nitrogen storing, there is also room for improvement in retaining the temperature of biospecimens while towers are lifted, see section 2.3.

Note that hardware to lift towers from liquid nitrogen tanks already exists, but they are either incorporated into fully automated storage systems or are only designed for specific “automation ready” storage systems. Our proposition explicitly aims to be applicable to all systems.

Condensing biospecimen tubes on SBS racks

For ecological and economic reasons storage space should always be used optimally. This means that there should not be empty positons on SBS racks, when they are put into long time storage (see step 4 in Figure 1). Therefore, biospecimen tubes are condensed on SBS racks before they are put into storage. This process of condensing tubes is especially challenging for SBS racks that will be stored in liquid nitrogen tanks at -196 °C. If the condensing is done by hand on dry ice at approximately -80 °C the difference to the desired long-time storage temperature is extreme. If the condensing is done in the vapour phase of liquid nitrogen, the vapour hinders the clear vision on the tubes. Altogether, in both settings, the very cold temperatures make it necessary to wear thick gloves for protection making it even harder to perform these fine-motor skills.

Developing a device that automatically condenses biospecimen tubes on two input SBS racks seems a worthwhile goal for the industry. Automated condensing devices already exist inside fully automated systems. Making them available as independent and smaller benchtop devices would close the gap between non-automated and fully automated systems as mentioned above. Thus, it would tackle the most crucial process step for biospecimens stored in liquid nitrogen.

Picking biospecimen tubes for selected retrieval

Analogous to the transfer of biospecimens into long-time storage, samples need to be handled individually as they are requested for retrieval by researchers (see step 7 in Figure 1). While they are condensed upon storing, typical retrieval requests usually comparing samples from different patient cohorts include only some of the samples from many SBS racks. Therefore, distinct tubes need to be picked from SBS racks.

In Figure 4 you can see a manual prototype of a device to support the cherry-picking process. With this prototype you need to identify the requested positions of the requested tubes first and manually place the metal pins in the device accordingly. Placing the whole SBS rack onto this device will then lift the respective tubes in the SBS

racks. This makes it manually easier to grab the tubes as well as minimizing the risks of mistakenly picking a wrong one.

Figure 4: Prototype of a manual picking aid (copyright Central Biobank UMG)

Optimizing this prototype, we propose an automated version that raises pins on its own, upon request. We envision this request being transmitted to the device via an interface from a laboratory information systems (LIMS). The samples are usually listed for retrieval in the LIMS anyway and with this interface, the picking aid would prepare the pins for the SBS rack at a moment's notice. This picking aid would optimally be operable in ultra-low temperatures and combined with a scanner to ensure, that the right rack is on top of the picking-aid.

This would increase efficiency and minimize the time biospecimens stay at warmer temperatures. It would be an equipment that could be added easily to any existing biobank setup.

2.3. Handling of biospecimens while maintaining ultra-low temperatures

Biospecimens move between different cooling devices during biobanking processes. These processes include transportation, scanning, storing, and retrieving (see steps 4 to 7 in Figure 1). Biobanks already strive to reduce the amount of time biospecimens are out of cooling devices as much as possible but there is still room for improvement.

Table 3 gives an overview of challenges regarding the handling of biospecimens while maintaining ultra-low temperatures.

Table 3: Summary of selected challenges of handling biospecimens while maintaining ultra-low temperatures.

Challenge	Possible Solutions	Maturity of solution
Insulation for storage towers from liquid nitrogen tanks	Develop an insulation surrounding the mechanic that lifts up towers from liquid nitrogen tanks (see section 2.2) to minimize the exposure to warm temperatures for the biospecimens in the lifted tower.	Idea
Removing ice from biospecimen tubes	Develop a device to automatically brush off ice using ethanol.	Idea
Scanning biospecimen tubes	Develop a scanner that can operate at ultra-low temperatures.	Idea
	Develop scanner with higher resolution, better illumination, lenses for optical zoom and generally improved scanning performance.	Idea

Insulation for storage towers from liquid nitrogen tanks

For example as explained in section 2.2 and shown in Figure 3 the tower of the liquid nitrogen tank is lifted to store or retrieve samples (see steps 4 and 7 in Figure 1).

While the tower is lifted all biospecimens above the position in question are exposed to warmer temperatures. Designing an insulation around the tower that reduces the heating rate of the biospecimens would be a valuable improvement.

Removing ice from biospecimen tubes

Upon arrival at the biobank (step 4 in Figure 1) and upon retrieval (step 7 in Figure 1) the unique data matrix code at the bottom of all biospecimen tubes is scanned, to confirm the identity of the handled tubes. This is a crucial step for quality control that cannot be skipped.

However, biospecimen tubes are transferred between environments with different temperatures and humidities and are possibly placed on dry ice. Some ice build-up on the samples cannot always be prevented. If there is ice on the data matrix codes of the tubes, these codes cannot be read by the scanners any more.

Ice buildup as in **Fehler! Verweisquelle konnte nicht gefunden werden.** makes a smooth scanning sequence impossible.

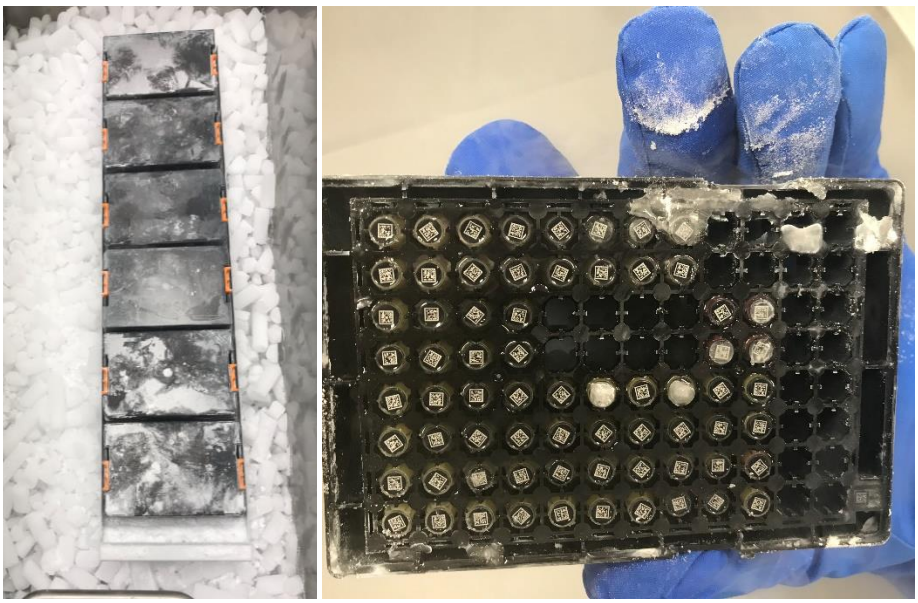

Figure 5: An example of SBS racks on dry ice (left) and a single SBS rack from below (right) with some apparent ice buildup.

Heating up the tubes is not an option lest the quality of the biospecimens be compromised. A common practice for biobanks is to use ethanol to melt off the ice from the tubes. This can be done manually but takes time and inevitably exposes the biospecimens to warmer temperatures.

We propose a device that automatically applies ethanol with a brush to the data matrix code of biospecimen tubes. Such a device would need to apply the brush with an appropriate amount of force to effectively clean the codes. The excess ethanol should also be cleared off in a second step. This should all happen at ultra-low temperatures.

Scanning biospecimen tubes

Even if all ice is removed from the biospecimen tubes, the scanning is not without challenges, as scanning usually takes place at room temperature (see Figure 6) or at -20 °C in automated systems. This is based on the inability of scanners to be used at ultra-low temperatures.

An approach for future work is to develop scanners that are insulated sufficiently to withstand ultra-low temperatures. Alternatively, designing box-specific insulation placed on top of the scanner to surround the SBS rack could reduce the heating rate of the biospecimens while at warmer temperatures on the scanner.

Another aspect of the challenge of scanning biospecimens (at warm temperatures) is the time it takes to get a decent scan result. Aside from the actual time it takes to get a single scan, it is often necessary to scan racks multiple times because the scan does not always deliver sufficient results upon the first try.

In order to tackle this problem and increase the performance of scanners, cameras with a higher resolution, brighter lights for better illumination of the data matrix codes and lenses with a better optical zoom could be deployed.

Figure 6: Rack scanner reading all 96 data matrix codes at the bottom of the tubes simultaneously.

3. Conclusion

As still very little is known about the effects of temperature fluctuations on sample quality and biobanks want to keep the possible future use of the collected biospecimens very broad, the best approach to maintain the quality unimpaired is to prevent interrupting the cold chain once the samples are frozen. This gold standard is challenged during several biobanking processes of which only a few were highlighted here.

The level of maturity of the solutions for these selected challenges to our knowledge varies. For the twelve introduced challenges, we presented solution approaches from which we categorized nine to be at least conceptual ideas and three even being available as prototypes. Many of the proposed solutions are fundamentally similar. They involve new mechanics, which together with the possibly necessary electrics should be well insulated when used in cryogenic temperature ranges.

For developing practical solutions here, close collaboration with engineering faculties or small and medium-sized enterprises would be helpful. The use of a network of biobanks, such as the German Biobank Alliance, to exchange information on new developments should also be sought.

However, a first step towards developing solutions is to summarize the existing challenges and communicate them to the developers.

4. Summary

In this work, three categories of cryogenic challenges encountered by biobanks to maintain the cold chains of frozen samples are described. For most of the challenges, at least conceptual ideas for solution approaches exist, in some cases even prototypes. A common theme between these challenges and solution approaches is a need for better insulation of mechanical parts to withstand ultra-low temperatures. To get fit-for-purpose solutions for their everyday challenges, it is critical that biobankers work closely together with (cryo)engineers to collaboratively develop solutions.

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