Review

Not peer-reviewed version

Evolution and Evaluation of Ultra-Low Temperature Freezers: A Comprehensive Literature Review

Christos Kypraiou and Theodoros Varzakas *

Posted Date: 29 May 2025

doi: 10.20944/preprints202505.2338.v1

Keywords: Ultra-Low Temperature (ULT) freezers; refrigeration technology; refrigerants; environmental sustainability; regulatory compliance; validation process; biological sample management; foods; healthcare; scientific research; energy efficiency

Review

Evolution and Evaluation of Ultra-Low Temperature Freezers: A Comprehensive Literature Review

Kypraiou, C. [1] **and Varzakas, T.** [1,2,*]

[1] Hellenic Open University

[2] University of the Peloponnese

* Correspondence: t.varzakas@uop.gr

Abstract: This review paper addresses the design and testing of Ultra-Low Temperature (ULT) freezers, highlighting their critical functions in various industries, particularly foods, medicine and research. ULT freezers operating at temperatures of -86°C and lower have come a long-way with improvements in freezing technology, for instance, from traditional vapor compression systems to new multi-stage refrigeration technologies. This progress has added operational reliability and energy efficiency, essential for preserving delicate samples and facilitating groundbreaking research. The article deeply explores the contribution of refrigerants to ULT freezer efficiency and sustainability. With the use of chlorofluorocarbons (CFCs), previously reliant on them, being prohibited due to environmental concerns, the sector opted for environmentally friendly substitutes like hydrofluorocarbons (HFCs), natural refrigerants, and hydrofluoroolefins (HFOs). Regulatory compliance is ensured by rigid validation protocols to guarantee ULT freezers are safe and meet quality requirements without compromising the integrity of the stored material. In addition to their wide-ranging advantages, ULT freezers also have disadvantages such as energy efficiency, incorporating automation, the integration of IoT and AI for proactive maintenance, and developing environmentally sustainable refrigerants. Adequate management strategies, including regular employee training and advanced monitoring systems, are vital to counteract threats from temperature variations and reduce long-term diminished performance. Finally, subsequent innovations in ULT freezer technology will not only aid in research and medical initiatives but also support sustainable practices, ensuring their core role as beacons of innovation in preserving the quality of precious biological materials and increasing public health gains.

Keywords: Ultra-Low Temperature (ULT) freezers; refrigeration technology; refrigerants; environmental sustainability; regulatory compliance; validation process; biological sample management; foods; healthcare; scientific research; energy efficiency

---

1. Introduction

Refrigeration and freezing systems play a critical role in various industries for which appropriate and precise management of temperature control is an essential function [1,2]. Specifically, the need for ultra-low temperature refrigeration has been more critical since 2020, following the emergence of the COVID-19 pandemic, which introduced new and complex requirements and demands for vaccine storage at temperatures of as low as -70°C (±10°C) [3,4]. Such temperature requirements require adopting more specialized solutions in the technology of refrigeration [5]. There is a variety of technologies available to meet modern demands, with the appropriate choice of the right technology being inextricably linked to the desired performance at temperature level and particular operational requirements [6,7]. For this reason, the need has fostered very impressive advancements of refrigeration technology for achieving superior energy and thermal efficiency and lesser temperatures for refrigeration [8].

Traditional refrigeration systems, such as the Vapor Compression Refrigeration (VCR) system, have also been widely utilized but are faced with enormous challenges to achieve Ultra Low

Temperature due to high compressor pressure ratios [6,7]. But whenever a high cooling capacity is required for bulk applications, air refrigerators are likely to be the preferred use since they can achieve high temperature efficiency, stability and uniformity [9]. These devices operate on the basic principle of energy conversion involving the use of a turbine to transfer energy from fluid, say air or steam, to mechanical energy, which can be used for various purposes, like power generation or cooling [9,10].

In contrast, for small-scale applications, piston compressor-based systems are becoming highly popular due to their affordability and reliability [11,12]. Piston compressors are highly reliable and effective due to their relatively simple design and ability to provide high pressure at an affordable cost [11,12]. In addition, they exhibit good performance over a variety of operating conditions, hence being popular in most applications [11]. They consist mainly of a cylinder and a reciprocating moving piston that compresses the gas inside the cylinder [11,12].

Among these systems, the ULT freezers are designed to remove heat from a cold tank using the least amount of energy usage, thereby maximizing power efficiency for specific cooling requirements [13–15]. ULT freezers are essential in areas that require extremely controlled low-temperature environments, such as biomedical, stability and scientific studies, electronics, chemistry, engineering, and several others [4,16]. In these industries, where precision temperature control is essential, ULT freezers ensure confidence of the stability and integrity of fragile materials and samples [17]. ULT freezers, which can achieve temperatures of up to -80°C (±10°C), are the secret to ensuring the stability and viability of highly temperature-sensitive materials, thereby enabling a wide range of applications from vaccine storage to genomics research [16].

The development of cooling technology has given rise to the development of ULT freezers, advancing from rudimentary vapor compression processes to sophisticated multi-stage refrigeration methods [4,18–20]. All this has been followed by huge advances in materials and engineering, allowing for more efficient use of energy and improved temperature consistency. The development of certain refrigerants, i.e., hydrofluorocarbons (HFCs) and natural refrigerants, has also accelerated the performance enhancement of modern ULT freezers, besides fostering better awareness towards environmental issues and sustainability [4,20]. The choice of an appropriate ULT freezer depends on serious consideration of various parameters and factors, e.g., temperature control precision, energy efficiency, and availability of space.

Given an increasing demand for regulatory compliance and validation in laboratory settings, organizations are now confronted with the challenge of choosing systems that not only fit their storage size but also meet demanding industry standards, recommendations, guidelines and strict regulations [21–23]. The demand for ULT freezers in research and biobanking presents the necessity to ensure that such systems offer reliable performance and maintain sample integrity over long spans [24–26]. While ULT freezers offer tremendous advantages such as maintaining sensitive samples and enabling path-breaking studies, they also bring along challenges that cannot be overlooked [19,27]. Energy consumption and maintenance requirements lead to operational expenses that affect the cost of laboratories and research institutions. Moreover, temperature excursions during equipment failure or power outages can cause irreversible destruction of valuable samples, necessitating robust monitoring and alarm systems to mitigate potential risks [4,20,28].

Thus, this paper attempts to critically review the technological dynamics and development of ULT freezers by investigating several key research aspects focusing from their history to new technologies, innovation and regulations. Firstly, it seeks to ascertain the developments in refrigeration technology that have governed the development of ULT freezers over the last few years, with a focus on transitioning from traditional systems to more complex methods that conform to greater levels of performance and energy efficiency. Secondly, the paper explores the impact of different refrigerants on the efficiency and environmental compatibility of ULT freezers, delving into the transition from ozone-depleting substances to eco-friendlier alternatives. It also distinguishes that compliance must meet various industries, using the requirement of rigorous validation to ensure that one can ascertain safety and quality. Moreover, the study focuses on advanced ULT Freezer

Technologies and a comparative analysis of those. Lastly, consideration includes the strength and weaknesses of using ULT freezers in high-stakes applications such as the medical and scientific research sector where temperature stability is most crucial. In general, the scientific world has recognized the importance of ultra-low freezers, not only as storage means, but also as a tool that maintains research and development [18,19,29].

With the right strategies, technologies, and processes, a sustainable future for storing sensitive samples that support advancement in science and healthcare can be created [18,19,30,31]. ULT freezers, if utilized together, combine science with social and health responsibility can result in a better and healthier society [18]. Innovation, participation and sustainable performance in health call for continued investment in storage technology [4,19,29]. Collaboration, regulatory compliance, and ethics will enable progress toward a healthier human and sustainable science [18,19,29–32]. Thus, by investigating and reviewing the above research aspects, this current review paper hopes to provide insight into the current status of ULT freezer technology and its significance for future developments in the field, reinforcing their value as precious sources for research and other related applications [4].

2. History

Lab freezers are specialized equipment designed to meet the rigorous requirements of scientific and medical application [33,34]. Even if they share the basic working principles of household freezers, by utilizing vaporized refrigerants, they are designed to yield much colder temperatures and with additional advanced features for high-accuracy temperature control, stability and uniformity within the chamber [35]. As the need for low-temperature preservation rose with the development of cryobiology in the 1940s and 1950s, researchers looked for ways to freeze and preserve biological materials without loss of viability [35,36]. Bioscience required not only freezing, but conservation at very low temperatures to maintain cell structure and viability [35,37]. At the midpoint of the 20th century, the first ultra-low-temperature freezers were released [35,38]. These adaptations of standard freezers utilized more advanced insulating materials and powerful refrigeration units [35,39]. The initial commercially produced -86°C freezers entered the market during the 1960s by manufacturers such as Thermo Fisher Scientific and Revco [35,37,40].

In particular, the discovery of cooling dates back to the 18th century when Scottish physician William Cullen was able to create a vacuum at lower temperatures [35,37]. Vacuum refers to the pressure of gases in the sense that it describes space where atmospheric pressure is reduced or essentially non-existent [41]. When a vacuum is generated, temperatures can drop as the gases expand and pull heat out of the surrounding environment [41]. This is the foundation for comprehending refrigeration and refrigeration processes [41].

Despite this, the application of refrigeration was first used practically in the 19th century through improvements like the vapor-compression refrigeration cycle, which was invented by Jacob Perkins in 1834, and which set the foundation for today's refrigerators and freezers [35,37]. Jacob Perkins developed the vapor-compression refrigeration cycle in 1834, a process by which heat is drawn from a material or environment using vapors [35,37]. The cooling process of refrigeration includes numerous primary steps. To begin with, there is compression during which a compressor compresses a refrigerant vapor, increasing its temperature and pressure. Second, this hot pressurized vapor subsequently moves into the condenser, where it releases heat to the environment and condenses into liquid [35,37]. Third, the liquid refrigerant subsequently moves through an expansion valve, which causes a pressure reduction and consequent cooling of the refrigerant [35,37]. Finally, the cooled liquid enters the evaporator, where it absorbs heat from the environment, evaporates once more, and continues the cycle. This cyclical process forms the basis of refrigeration system functioning, allowing effective cooling [35,37].

The process formed the foundation for modern refrigerators and freezers, whereby food and other products can be stored in cold temperatures [16,36,38,42,43]. The development of this technology has had a significant impact on the food sector, transportation and storage commodities [16,36,42,43]. Finally, during the beginning of the 20th century, advances in refrigeration technology

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 May 2025 doi:10.20944/preprints202505.2338.v1

continued to result in the emergence of mechanical refrigeration systems that were capable of achieving lower temperatures [16,36,38,43]. These machines were first used in commercial environments, such as meat processing factories and breweries [16,36,42].

During the last few decades of the 20th century and early 21st century, monumental technological advancements improved the usability, reliability, and effectiveness of ULT freezers [28,34,35]. These developments have assisted in safely and efficiently storing valuable materials and samples [34,44]. Among significant innovations was the insulation [45,46]. Over the decades, insulation products such as polyurethane have emerged as the norm, enhancing energy efficiency, efficient vacuuming and temperature management [45,46]. The lack of frosting accumulation has also been a measurable advantage, reducing the maintenance frequency [45,46]. Additionally, the application of microprocessors in freezer systems during the 1980s brought a new era in the monitoring and control of temperature [35,36,47,48]. These sophisticated systems preserved samples in the required range of temperature and also had alarm functions for excursions into protective service of valuable materials [35,36,48].

With respect to energy efficiency, the demand for cleaner technologies has been motivating the development of energy-efficient ULT freezers [35,36,48,49]. Research into alternative refrigerants and more effective compressor technology has far reduced the environmental impact of such appliances [35,36,48,49]. Additionally, modern ULT freezers also come equipped with digital monitoring systems that provide real-time temperature control, along with internet remote access [48,50]. Technology is used to deliver maximum protection to precious samples such that there will be a speedy response to any complications [48,50]. For maximum ease of use, accessibility, and ergonomics, freezers have been made easy to use, maintain, and clean [48,50,51]. Features such as adjustable shelves and easy-to-read displays make them ultimate user-friendly machines for maximum use in day-to-day practicality and convenience in their operations [28,52].

With time, the refrigerants used in ULT freezers have gone through significant evolution due to environmental concerns and policy developments [50,51]. Perhaps the most important evolution is the phasing out of chlorofluorocarbons (CFCs) by hydrofluorocarbons (HFCs) as the major refrigerants [53–55]. This transition is one of the bigger changes in environmental policy and technological adaptation to reduce harm to the world [53–55]. Chlorofluorocarbons (CFCs) were initially developed in the early 20th century and shortly became extremely popular as refrigerator propellants due to the fact that they were stable, non-flammable, and efficient in terms of energy [56,57].

Labs and a wide industry applied CFCs in the air-conditioning and refrigeration processes [56,57]. In the middle of the 20th century, however, scientific research indicated a serious environmental issue, CFCs were very destructive to the stratospheric ozone layer [54]. The ozone layer is critical in protecting life on Earth by degrading most of the sun's deadly ultraviolet radiation [56,57]. Research in the late 1970s and 1980s showed that CFCs, once released into the atmosphere, would break down and release chlorine atoms [53–55]. These chlorine atoms subsequently caused the depletion of the ozone layer, creating primarily the annual Antarctic ozone hole [53–55].

In response to the growing environmental risk caused by CFCs, countries from all around the world came together and signed the Montreal Protocol in 1987 [56,57]. The first-ever environmental treaty aimed at phasing out the use and manufacture of ozone-depleting substances like CFCs [53,54,57]. The protocol sets binding reduction targets on the consumption and production of CFCs, encouraging industry and researchers to identify safer substitutes [53,54,57].

With phase-out of CFC, hydrofluorocarbons were the major substitute [53,54,57]. HFCs are non-chlorine-containing and therefore do not deplete the ozone layer, which made them a sought-after substitute [53,54,57]. Their chemical composition allowed efficient regulation of heat, which made them ideal for laboratory freezers that require precise control of temperature to preserve sensitive samples [53,54,57]. Although HFCs have severed the problem of ozone depletion, new environmental problems have been created [53,57]. HFCs are potent greenhouse gases that possess high Global Warming Potential (GWP) [50,53,54,58].

As much as climate change issues have been solidified, the environmental impacts of HFCs have fallen under control [53,54]. In turn, the industries and governments are actively searching and investing for creating and using more environmentally friendly refrigerants with lower GWP but still maintaining the level of functionality needed for crucial laboratory and industrial uses [53,55,57]. The transition from CFCs to HFCs is an essential step in the development of cooling technology to address environmental needs [53,55]. In the future, studies will persist in the innovation of refrigerants that are efficient and environmentally sound [55]. Alternatives such as hydrofluoroolefins (HFOs), natural refrigerants such as ammonia or CO2, and emerging technologies remain in focus as being sustainable, sustainable substitutes for HFCs [53,55].

The development of ULT freezers is a testament to scientific and engineering advancements [35,37,55]. From the inception of refrigeration methods to recent advances in ULT technology, the freezers have become vital devices for the maintenance of biological integrity and the success of scientific inquiry [35,37]. With further developments in technology, future ULT freezing has even more effective, sustainable, and inexpensive systems to further optimize their applications in research and industry [35]. Overall, the history of refrigerants used in laboratory freezers is a mirror of the greater challenges and advancements of achieving a balance between technology needs and environmental management [37]. With increasing global priorities for sustainability, the refrigeration industry will continue to change, seeking refrigerants with minimal environmental impacts since they aid the essential roles of scientific research and medicine [35,37].

3. Refrigerants

The past decades have seen the air conditioning and refrigeration industries facing a serious challenge that is balancing performance with efficiency and environmental responsibility [58–60]. This issue largely stems from the regulation of high global warming potential (GWP) refrigerants, which has spurred looking for alternatives [58–61]. In terms of how the refrigerants work, basically, the refrigerant enters the compressor as steam, is compressed into hot liquid [1,62,63]. The liquid passes through the condenser, losing some of the heat but still in liquid form. It then goes through the coils of the evaporator, where a reduction in pressure causes it to revert to its gaseous state [62–64]. The process absorbs heat from the environment, thus cooling the interior of the freezer [60,62–66]. Considering growing concerns over climate change and ozone depletion, there is a necessity to research various refrigerants, their characteristics, strengths, and weaknesses [60,62–66].The current section strives to provide an overview of the main refrigerant classes, including hydrofluorocarbons (HFCs), hydrocarbons, ammonia, carbon dioxide, hydrofluoroolefins (HFOs) and specialty refrigerants, and to evaluate their impact on sustainability and environmental safety [58–60,67]. A summary of the following analysis is presented at Table 1 below.

Hydrofluorocarbons (HFCs) have been widely used since the end of the 20th century as a replacement for ozone-depleting substances, e.g., chlorofluorocarbons (CFCs) and hydrochlorofluorocarbons (HCFCs) [58–60]. They have high thermodynamic properties and find broad applications ranging from commercial refrigeration to air conditioning [58–60,63]. They have a critical negative characteristic which is their high GWP [60,63]. Transitioning away from HFCs is a vital part of the fight against climate change and has led to the quest for more eco-friendly sources of refrigerants [58–61,63].

R-134a (tetrafluoroethane) is one of the most widely used HFCs in refrigeration [58,66,68,69]. It is highly efficient and available with respect to energy and can be used for both low and medium temperature applications [66,68]. Despite this, its high GWP makes it not a desirable option to continue [58,66,68,69]. Moreover, R-404A is also a blend of HFC-125, HFC-143a, and HFC-134a [70]. This mixture is suitable for its cooling capacity, and while it is strong during low temperatures, it also possesses a high GWP [59,64,66,71,72]. The industry is gradually reducing the application of R-404A as efforts are made to eliminate high GWP refrigerants [59,64,66,71,72].

In addition, R-407C, made up of HFC-32, HFC-125, and HFC-134a, serves as a replacement for R-22 [59,72–75]. It possesses a lower GWP than some traditional HFCs but is still not the best

doi:10.20944/preprints202505.2338.v1

environmentally with its increased GWP over new alternatives [59,72–75]. Secondly, R-410A is another blend (HFC-32 and HFC-125), which is used for its high refrigerant charge and low noise [59,72–75]. As in the case of other HFCs, it is regulated under environmental laws based on its high GWP [59,62,65,76,77]. Finally, R-507A, a mixture of HFC-125 and HFC-143a, demonstrates good performance in low temperatures but suffers from the same environmental concerns regarding high GWP [60,62–66].

Hydrocarbons, including propane (R-290), isobutane (R-600a), and ethane (R-170), have been progressively utilized to replace HFCs since they are characterized by low GWP and high energy efficiency [14,78,79]. However, flammability characteristics of hydrocarbons require strict safety measures when in storage and utilization [14,78]. Whereas hydrocarbons are a promising avenue for green cooling technology, the hazards associated with them make stringent safety measures necessary [14,78,80,81].

R-290 (propane) is also lauded for having much less GWP than HFCs and has proven to be an acceptable substitute for use in refrigeration [71,81–84]. It has high energy efficiency as a significant advantage but, on the other hand, is flammable and poses problems that must be treated carefully [71,81–84]. R-600a (isobutane), meanwhile, is another eco-friendly option with low GWP. It has good performance and efficiency but, like R-290, it requires special safety measures since it is a flammable material [58,78,81,85,86]. In addition, R-170 (ethane) is described as having very low GWP and efficiency but a narrow range of application and flammability, thus limiting its use in practice [14,79,80,87].

Ammonia (R-717) is a high-performance refrigerant with a long history of use in large industrial refrigeration systems [88,89]. One of its best features is that it is extremely cheap and highly effective [88,89]. It is extremely effective in the cooling procedure and is capable of supplying much greater cooling capacity compared to most of its alternatives [88–90]. However, the toxicity of ammonia requires stringent safety standards [88,89]. Ammonia causes far less environmental harm compared to HFCs and is hence widely utilized for bulk uses such as food processing refrigeration and refrigeration plants [103,168]. However, due to its toxicity, ammonia systems should be well planned in design and operation to prevent any potential health risks [88,89]. Its toxicity has tight safety precautions while handling it, which necessitates the training of workers and facilities to fit in detection systems and safety locks to prevent accidents [88,89].

Carbon dioxide (R-744) is an outstanding refrigerant option, with low GWP, non-flammability, and non-toxicity, and a natural refrigerant [60,67,91–95]. Its physical characteristics, e.g., high operating pressure, require special equipment for safe use and efficient functioning [60,92,94]. CO_2 is also increasingly being used in many applications, e.g., supermarket refrigeration, due to its favorable environmental balance [60,92,94]. Nevertheless, CO_2 systems are also susceptible to the limitations of needing to employ high-technology equipment and additional up-front investment capital since equipment used to work with high pressures is sophisticated [60,92,94]. Carbon dioxide is an attractive option as business moves towards greener approaches [60,92,94].

Hydrofluoroolefins (HFOs) synthesized because of regulatory directions against high GWP molecules are a next-generation refrigerant that attempts to reconcile the best thermodynamic features of HFCs with extremely low GWP values [96–98]. HFOs display a promising move towards environmentally friendly refrigerants but are hampered by infrastructure readiness and the need for further studies on their long-term environmental implications [96–98].

R-1234yf (2,3,3,3-tetrafluoropropene) is the most recognized HFO, which has been approved for use in refrigeration systems in motor vehicles and for some commercial uses [86,96,98–100]. It possesses a GWP that is about 75% less than that of R-134a, and it is thus a superior environmentally friendly replacement [86,96,98–100]. Yet, some drawbacks come along with this refrigerant, such as its comparative novelty, which leads to a lack of availability and the necessity of new infrastructure and/or qualifications [86,96,98–100]. Moreover, R-1234ze (Trans-1,3,3,3-Tetrafluoropropene) is another low-GWP HFO applied in various refrigeration and air conditioning applications [51,96,101–103]. Similar to R-1234yf, it has faced regulatory scrutiny and is widely considered as a replacement

 doi:10.20944/preprints202505.2338.v1

for higher GWP refrigerants [51,96,101–103]. Procurement and technology infrastructure constraints for both R-1234yf and R-1234ze are significant issues when firms transition to such newer pairings [51,96,101–103].

The need for environmentally friendly and energy-efficient refrigeration technologies is driving the increased adoption of natural refrigerants [88]. There are three major natural cooling taps which are butane (R-600), carbon dioxide (R-744), and ammonia (R-717) [42,95]. R-600, also known as butane, is a natural refrigerant that has garnered attention due to its low global warming potential (GWP) [78]. Its capacity for good refrigeration makes it best suited for numerous applications, ranging from household freezers and fridges [42,78]. However, its flammability requires caution in its handling and storage [78]. This implies that facilities employing the use of butane will be required to adhere to certain safety requirements to reduce the chances of accidents [42,78]. Generally, R-600, R-744, and R-717 natural refrigerant taps possess some pros and cons that must be addressed while choosing the right refrigerant for specific applications [42,88,95]. While butane is a cheap and energy-efficient option, carbon dioxide and ammonia offer innovative solutions in environmental conservation and energy efficiency, as long as proper safety measures are used [42,88,95]. The choice of the right refrigerant goes a long way to determine the efficiency and sustainability of the refrigeration system, and thus the selection process becomes crucial for modern-day refrigeration industries and applications [42,88,95].

Apart from the primary refrigerant varieties, certain specialty blends and less common refrigerants have appeared in various applications [42,104–106]. These blends typically target specific performance characteristics or specific applications [42,104,105]. It is to be noted that continuous research to find effective refrigerants is not only aimed at finding those with low GWP but also to make them have adequate cooling performance and safety parameters required for various applications [42,104–106]. R-401A is a mixture of HFC-125, HFC-143a, and HCFC-22, employed primarily as a substitute for R-22 in some systems [42]. Although it is sufficient in its capabilities, its GWP rating is high, so it is viewed as a disruptor rather than something that will endure [124]. R-421A is an azeotropic blend that aims to replace R-22 with a reduced GWP [42,75,101]. There are still, however, supply issues, making its global acceptance and use in present installations difficult [105]. R-40 (Ethylene) and R-12 (Dichlorodifluoromethane) are among the phased-out refrigerants due to their toxic environmental impacts, such as ozone depletion and high GWP [104,106,107]. Nevertheless, in certain specialized applications, R-40 is recognized for its low GWP and non-toxicity but with restricted applications [104,106]. The shift from HFCs to an eco-friendlier group of refrigerants is critical in addressing the years-long pursuit of solutions to climate change and ozone depletion [59,68,72]. Though HFCs are good refrigerants, their elevated GWP calls for an irreversible shift towards alternatives with a lesser environmental impact [81–84].

Hydrocarbons, ammonia, carbon dioxide, and HFOs are some of the pioneers in this transition, each of them having certain advantages and disadvantages [42,95,102]. Hydrocarbons are renowned for their environmental benefits but are tempered by safety concerns related to flammability [88,89]. Ammonia stands out as being both effective and affordable for industrial applications [71,81–84]. Toxicity creates threats that must be treated with utmost caution and protective protocols [42,95,102]. Carbon dioxide is a wonderful alternative with a superb environmental track record, albeit availability is subject to high-technology deployment [88,89]. HFOs are the new wave of innovation, coupling improved environmental standing with existing applications but deployment may be delayed by infrastructure constraints and regulatory uncertainty [88–90].

In general, the continuous innovation and adoption of alternative refrigerants rely not only on their environmental features but also on technological developments, policy regulations, and market readiness [88,89]. Efficient transition to such green alternatives is imperative in reducing the carbon footprint of the refrigeration and air conditioning industries and, in turn, contributing to greater environmental goals [42,95,102]. All steps taken in this transition must be backed by careful planning, investment in new technologies, and emphasis on safety such that the benefits of adopting new refrigerants are obtained without undesirable impacts [42,95,102]. The refrigeration industry stands

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 May 2025 doi:10.20944/preprints202505.2338.v1

today at a critical point in its history, and choices today will affect future generations and the well-being of our world [42,95,102].

Table 1. Types of Refrigerants.

Group	Type	Attribute	Advantages	Disadvantages	References
Hydrofluorocarbons (HFCs)	R-134a	Tetrafluoroethane	Good energy efficiency, Widely available	High GWP, contributes to climate change	58,66,68,69
	R-404A	HFC-125, HFC-143a and HFC-134a blend	Cooling capacity, Good performance at low temperatures	High GWP, under acceptance	59,64,71,72
	R-407C	HFC-32, HFC-125 and HFC-134a blend	Replacement of R-22, Lower GWP	Higher GWP than other solutions	59,72-75
	R-410A	HFC-32 and HFC-125 Blend	High Cooling Capacity, Low Noise	High GWP, phased acceptance phase	59,72-75
	R-507A	HFC-125 and HFC-143a mixture	Consistent performance at low temperatures	High GWP	60,62-66
Hydrocarbons	R-290	Propane	Significantly lower GWP, Energy efficient	Flammable, requires precautions	71,78,79,81,83,84
	R-600a	Isobutane	Low GWP, Good Performance	Flammable, requires precautions	58,78,81,85,86
	R-170	Ethane	Very Low GWP, High Efficiency	Flammable, limited applications	14,79,80,87
Ammonia	R-717	Ammonia	High Efficiency, Low Cost	Toxic, requires safety precautions	88,89
Carbon Dioxide	R-744	Carbon Dioxide	Low GWP, non-toxic and non-flammable	High operating pressure, requires special equipment	60,67,91-95
Hydrofluoroolefins (HFOs)	R-1234yf	2,3,3,3-Tetrafluoropropene	Low GWP	New, limited availability and infrastructure	86,96-100
	R-1234ze	Dia-1,3,3,3-tetrafluoropropene	Low GWP	New, limited availability and infrastructure	51,96,101-103
Natural Cooling Taps	R-600	Butane	Low GWP, good cooling properties	Flammable, requires precautions	42,95
	R-744	Carbon Dioxide	Low GWP, non-toxic and non-flammable	High operating pressure, requires special equipment	60,67,91-95
	R-717	Ammonia	High Efficiency, Low Cost	Toxic, requires safety precautions	88,89
Special Cooling Taps	R-401A	HFC-125, HFC-143a and HCFC-22 Blend	Used as a replacement for R-22	High GWP, not a viable solution in the long term	42
	R-421A	Azeotropic mixture to replace R-22	Lower GWP than R-22	Limited availability	42,75,105
Inert Gas Refrigerant Taps	R-40	Ethylene	Low GWP, non-toxic	Limited Apps	104,106

Group	Type	Attribute	Advantages	Disadvantages	References
Other Cooling Taps	R-12	Dichlorodifluoromethane - has been withdrawn due to ozone depletion	Cooling capacity at low temperatures	High GWP, causes ozone depletion	104,106,107
	R-22	Chlorodifluoromethane – withdrawn due to HFC regulations	Good performance and wide use	High GWP, withdrawn due to HFC regulations	42,59,72-75

4. Importance and Application of ULT Freezers

Laboratory freezers are classified based on their temperatures as moderate (-20°C), ultra-low (-86°C), and cryogenic (<-150°C) [59,82,108]. Precisely, storage between a temperature range of -20°C to -150°C is normally used for special purposes mainly in scientific and laboratory use, and industrial sectors [78,89]. Freezers are categorized based on their operational temperature ranges, each with special applications suited to various storage needs. Deep Freezing Freezers (operating between -50°C and -20°C) are employed in the storage of sensitive biological products such as blood samples and vaccines [109]. These products must be kept under constantly low temperatures in order to maintain their stability and effectiveness [60,72,99,108]. On the other hand, ULT Freezers, which work at between -86°C and -50°C, play a critical role in maintaining biological samples, cells, proteins, and other substances intact for extended periods of time. Such a freezer is largely utilized in biotech and the pharmaceutical sector where sample conservation is of crucial importance to ongoing research [64,68,91,108]. Finally, Cryogenic Freezers operating at temperatures below -100°C are utilized for high-end cryogenic applications. They play an important role in the storage of human cells, stem cells, DNA, and RNA, which need to be preserved at extremely low temperatures so that they remain alive and in intact form [13,110,111] Thus, storage at ultra-low temperatures is a crucial factor in pharmaceuticals, biomedicine, food storage, and research fields, protecting the integrity and efficacy of various products [1,32,112,113]. Since technology has progressed at a rapid rate, effective methods of storage, particularly sensitive materials such as pharmaceuticals, biological samples, and foods, have been in higher demand [1,112,114,115]. Table 2 categorizes these products and their optimal storage temperatures, a factor which reflects their unique need for preservation [112–115].

In the days to come, ULT freezer technology can do much more. The combined technology of high-temperature control and its integration to a considerable extent in many different research fields will come back with huge dividends [27,116,117]. Digital monitoring capability, automation capacity, and the development of smart cooling facilities will be required in the future to enable the best possible conditions for storage and preservation [47,116,118]. In the current era, ULT freezers ought to be able to keep up with the most rigorous regulations and address the increasing needs of the scientific and research world [116,118]. Focus on investment and research in the development of highly advanced technologies and recognition of the importance of preservation of precious samples will maintain ULT freezers fashionable with scientific progress [27,116].

The success of modern medicine and food research also mostly depends on the ability to store sensitive samples in extreme conditions [116]. Not only do ULT freezers provide the assurance of safe and long-term storage, but also provide the ability to develop new drugs, vaccines, and therapeutic protocols [118–120]. The progress of medical research, such as gene therapy and immunotherapy, requires stable and reliable storage of histological samples and biological materials to maintain their functionality [116,117].

Since global health crises are an ongoing reality, research and development will be required in order to prepare and effectively arm the medical community [116–118]. In addition, the expansion of vaccine and drug supply, particularly during global health crises, highlights the significance of ULT freezers [4,121,122]. Temperature control and storage conditions may result in the stability of vaccines, which has been well illustrated in the recent COVID-19 pandemic [3,4,123]. Clinical trials and documentation of results processes require stringent and rigorous storage protocols, which are supported by the proper utilization of ULT freezers [121,122].

With evolving technology, the ULT freezer industry tends to implement innovation that reduces the consumption of energy and maximizes usability and security [116]. Advances in refrigeration technology, for instance, the use of more efficient refrigerants, integrated monitoring solutions with IoT (Internet of Things), and artificial intelligence solutions to forecast needs and control temperature are most likely to have a gigantic impact on the handling of research and samples [124]. Also, with a more globalized world, supply chain efficiency in delivering far-traveling samples requires safe and reliable cooling systems [47,116,124]. ULT freezers must be able to withstand several man-made and environmental factors at high speeds and in an efficient capacity so that there is a consistent and stable storage condition wherever these sensitive materials are being transported [116,118].

Government and global collaboration can also form a significant part of the creation of regulations surrounding the operation of ULT freezers [28,52]. Such collaboration can be in the form of setting common regulations or standards for sample storage and transportation [24,52,86]. The "World Health Initiatives" and the "World Health Organization" are some of the global organizations that can assist in cementing the regulatory process that will ensure the ongoing safety and integrity of samples across the world [18,125]. Moreover, data collection and analysis concerning the stability and efficacy of samples in ULT freezers will probably be among the most influential factors on the entire process of new therapeutic strategy development [24,52,86]. The availability of good and current data regarding sample behavior when stored may be of significant worth in offering insights into the pressing need for qualitative and quantitative improvement in storage processes [24,52,76,86].

ULT freezers are governed by national and international healthcare policy [76]. Unlimited government agency funding for healthcare and research can enhance the security of samples and the preparedness of many industries to respond to abnormal and critical events [52,76,86]. Policy formulation for ensuring the promotion of research and regulation of ULT freezers is necessary in a bid to ensure the response to any emergency [116,124]. For example, in healthcare, ULT freezers are increasingly becoming the cornerstone of an expanded interrelation between technology innovation, and health [116,124]. These products not only meet basic needs but are also central to new treatments and public health preservation [18,125]. Organizations are encouraged to estimate the value of this west, both at the resources and at the research and development stage, in an attempt to enhance their ability to compete in a more changing environment [18,52,76,86,125].

ULT freezers are critical for the storage and handling of temperature-sensitive materials that require close stability for research and other scientific purposes [13,59,82]. Proper handling, temperature wide range and use of these freezers are crucial for the success of a variety of samples, food, and research [13,82,110]. Users can set specific temperatures, ensuring these essential elements are kept within set parameters [59,82]. Moreover, to protect against unexpected temperature fluctuations from power loss or equipment malfunction, laboratory freezers in most laboratories have internal alarm systems and backup cooling systems on the basis of CO_2 or N_2 [58–60]. These elements ensure continuous monitoring and automatic response to protect stored material [58–60]. Explosion-proofed construction through the avoidance of combustible materials and locking devices can also contribute to security and safety [58–60,63,64]. Briefly, freezers in laboratories are precious equipment to the scientific world, providing invaluable support in the preservation of delicate materials [60,62–66]. Their advanced features, ability to operate under extreme temperatures, and protective measures make them considerably superior and advanced compared to domestic models [60,63,64].

Microbiology is based on ULT Freezers to store microbial cultures and other biological reagents [114,115,126]. Crops need to be stored at lower temperatures in order to maintain their viability and enable future experiments [113,126,127]. Biologists and microbiologists need their samples to be stored in a safe place so that they can maintain the crops safe from losing vital biological characteristics as well as their growth capability [113,127]. Moreover, ULT freezer makes it possible for microbial culture to be stored for long periods without loss of its biological activity [113,126,127].

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 May 2025 doi:10.20944/preprints202505.2338.v1

The ability to maintain crops under temperatures of zero by a margin provides the ability to conduct experiments with reliable results, which are crucial to the invention of new drugs, vaccines, and biotechnological products [113,115,127].

Apart from that, ULT freezers allow researchers to conserve microbial and plant species genetic diversity, thus making evolutionary biology and ecological research possible [114,115]. Such applications not only augment understanding of biological processes but also allow researchers to acquire essential evidence of biodiversity and natural resource conservation [114,126,127]. Additionally, pharmacogenetics and genetic research are also significant areas where ULT freezers are now a necessity [1,32,82,111,112]. Pharmacogenetics is more relevant today than ever before, since the process of tailoring treatments to fit unique genetic patient profiles is becoming more and more developed [1,32,82,111,112]. ULT freezers create the conditions for continuous preservation of genetic material, which forms the basis of personal medicine, wherein patients are treated more effectively and safely [1,32,82,111,112]. Freezing and genetic research is a foundation of discovering new treatments and understanding genetic elements that influence disease and health [1,32,82,111,112,128]. ULT freezers are crucial in DNA, RNA, and other genetic materials preservation, with strict temperature regulation to prevent spoilage [1,32,82,111,112,128].

Genetic samples have to be preserved to conduct experiments and obtain results that are valid [26,32]. These samples may be from studies, clinical trials, or biobanks and are likely to require storage conditions that ensure their full integrity [26,32,112]. But apart from storage, ULT freezers also help to analyze such samples so scientists may perform complex experimental procedures such as PCR (polymerase chain reaction), DNA sequencing, and transcriptome analysis [32,82,111,112]. This research furthers the understanding of the mechanisms of genetic diseases and drug resistance, thus facilitating the implementation of supportive measures towards the success of medicine and pharmacy [32,82,111,112].

Pharmaceuticals are a general class of drugs that can be either small molecular agents or biologics [1,32,112]. Smaller molecular compounds are typically stored at temperatures ranging from -20°C to -80°C [1,32,112]. Storage at these temperatures is required to prevent degradation and drug effectiveness [1,32,112]. Organic substances, such as peptides and proteins, often require even more critical storage conditions, -80°C to -20°C, depending upon composition [112]. Changes in temperature can lead to loss of activity or degradation, highlighting the importance of controlled environments [1,32,112].

The class of large molecules is primarily composed of biological products such as monoclonal antibodies, recombinant proteins, and viral vectors [114,126,127]. Monoclonal antibodies are a pillar in therapeutic treatments, such as cancer treatment. They are kept at a temperature of -80°C to -20°C to maintain their structural organization and function [113–115]. Recombinant proteins are also part of this category with the same temperature requirements [115,127]. Their complex structures are also vulnerable to temperature changes, and improper storage can lead to significant loss of activity [113–115,126]. Gene therapy-applied viral vectors are extremely sensitive and require storage at -80°C or liquid nitrogen at -196°C to be able to transport genetic material efficiently [114,115,127].

Storage of biological samples is of utmost importance for research, clinical and diagnosis purposes. Like DNA and RNA, they need temperatures ranging from -196°C to -80°C to avoid decomposition [44,123,129–131]. The preservation of nucleic acids is the most crucial aspect for accurate results in gene analysis [44,123,132]. Proteins, used in various research studies, are to be kept between -80°C and -20°C temperatures to extend their shelf life as well as maintain their functional properties [44,123,129–132]. Similarly, vaccines, and more so mRNA technology-based vaccines, are an area of priority in public health [34,117,133]. Most vaccines need to be kept at -80°C to -20°C, whereas mRNA vaccines are specifically kept at a minimum of -80°C to -60°C achievable, which shows their deliquescence state and heat sensitivity [34,117,133]. These storage requirements are critical to vaccine effectiveness and avoidance of potential side effects [34,117,129,133].

Transfusions and medical procedures are reliant on blood products [2,134–138]. Blood is preserved at -80°C or lower, and platelets are preserved at 4°C for short-term use but -80°C for long term [2,134–138]. The variation emphasizes the importance of proper storage conditions to maintain available safe and effective blood products, in accordance with the requirement to ensure quality and safety across the complex process of storage and management [2,134–138].

The topic of cell culture encompasses a wide variety of cell types that require varying storage conditions [127,139–141]. Inhibitory cells, cell lines, and stem cells, for example, usually have abnormally low storage conditions between -196°C and -80°C [127,139–141]. This indicates the cell sensitivity of viable cells, where incorrect storage would lead to cell death or loss of viability [127,139–141]. Moreover, it is necessary to conserve tissue samples for the purposes of histological analysis, pathology, and medical research [142–144]. Fresh frozen tissue samples and other tissue samples are best stored between -196°C and -80°C temperatures, maintaining cell architecture along with biological function for future analysis [143,144]. Preservation like this helps researchers and doctors diagnose properly and investigate pathological conditions [142–144].

The genetic material includes DNA plasmids and oligodynamic, which are widely used in molecular biology [145–147]. The materials must be stored at stable temperatures ranging from -196°C and -80°C to prevent decomposition and provide reliable experimental results [145–147]. Authenticating the integrity of this genetic material is vital to the accuracy and credibility of findings [145–147]. Moreover, for environmental samples, storage at -80°C is recommended to preserve their physical properties and prevent them from being biologically or chemically altered [148–150]. The same applies to clinical samples, where storage at -80°C preserves the stability and integrity of biological analysis [148–150]. Experimental observations, however, require greater flexibility in storage from -196°C to -80°C depending on the samples type and the specific requirements of the experiments [148–150]. These storage conditions allow for samples to be stored in optimal conditions for further analysis and processing [148–150].

Lastly, storage temperature for short shelf-life foodstuffs like frozen fruits, vegetables, meats, fish, milk products, and prepared dishes tends to be between -40°C and -18°C, varying with product type and storage conditions [12,35,41,48]. While standard commercial refrigerators range from -30°C to -18°C to ensure food safety and preserve quality, ultra-low temperature refrigerators can maintain storage in the -40°C range for long-term preservation, specifically for sensitive items like some fruits and vegetables [12,35,41,48]. Maintaining the refrigerated conditions slows down microbe growth and enhances enzymatic activity, thereby enhancing shelf life while preserving the nutrients within accessibility [12,35,41,48]. However, daily use of ultra-low freezers for food storage is less frequent since regular freezers are sufficient for most food storage needs [12,35,41,48]. Proper temperature control is essential to prevent spoilage, ensure safety, and preserve flavor and texture when food is stored for long periods of time [12,35,41,48].

As technological advances continue, improvement in storage devices and surveillance systems will be increasingly crucial across all industries but particularly in biotechnology and pharmaceuticals, which require high performance from products [121,148–150]. In total, beyond their technical function, the success concerning the integrity and security of samples stored in these freezers has additional implications [18,52,76,86,125]. ULT freezers not just store valuable samples, but also make advancements in science possible, creating a bridge of mind between modern medicine and future discoveries that can change the trajectory of human medicine. Investment in ULT freezers is therefore a strategic choice with direct implications for the ability of the scientific community to generate decisions and innovations that will improve the quality of life and health of the population [116,124]. Continued growth and innovation in the field of such industries and research work may determine the future treatment and solutions that will address the health care problems of the future [18,52,86,116,124]. ULT freezers are not just storage equipment. They are beacons of innovation, lighting the way to breakthroughs that can save lives and transform the lives of tens of millions of individuals across the globe [18,52,86,116,124]. The importance attached to low-temperature technology reflects the close association between science and society, as scientific inquiry and development continue to impact public health and welfare [18,52,76,86,116,124].

 doi:10.20944/preprints202505.2338.v1

Table 2. Product Items & Storage.

Category	Product Type	Standard Storage Temperature at Extremely Low Temperatures (ULT)	References
Pharmaceuticals	- Small molecular drugs	-80°C to -20°C	1,32,112
	- Organic (e.g., peptides)	-80°C to -20°C depending on stability	1,32,112
Large Molecular / Biological	- Monoclonal antibodies	-80°C to -20°C	114,126,127
	- Recombinant proteins	-80°C to -20°C	113-115,126
	- Therapeutic proteins	-80°C	113-115,126
	-Enzymes	-80°C to -20°C	12,35,41,48
	-Viruses	-80°C or -196°C	64,68,91,10
	- Antibodies against RNA lines	-80°C to -20°C	44,123,132
Biological samples	- DNA/RNA	-196°C to -80°C	145-147
	-Proteins	-80°C to -20°C	64,68,91,108
Vaccines	- mRNA vaccines	-80°C to -60°C	34,117,133
Blood products	- Blood	-80°C or cooling (<4°C)	2,134-138
	-Platelets	4°C for short-term storage; -80°C for long-term storage	2,134-138
Cell Culture	- Cell lines	-196°C to -80°C	127,139-141
	- Fetal/stem cells	-196°C to -80°C	127,139-141
Tissue Samples	- Fresh frozen samples	-196°C to -80°C	143,144
	- Paraffin (formalin) samples	-80°C to -20°C (for long-term storage)	143,144
Genetic material	- DNA plasmid	-80°C	145-147
	- Oligodynamic	-80°C to -20°C	145-147
Research samples	- Environment samples	-80°C	148-150
	- Clinical samples	-80°C	148-150
	- Experimental observations	-196°C to -80°C	148-150
Food (Perishables)	- Frozen fruits and vegetables	-40°C to -20°C (for long-term storage; in ultra-low freezers)	12,35,41,48

 doi:10.20944/preprints202505.2338.v1

Category	Product Type	Standard Storage Temperature at Extremely Low Temperatures (ULT)	References
	- Meat and seafood (frozen)	-30°C to -18°C	12,35,41,48
	- Dairy products (frozen)	-30°C to -20°C	12,35,41,48
	- Ready-to-eat frozen meals	-30°C to -18°C	12,35,41,48

5. Advantages and Challenges of ULT Freezers

ULT freezer usage is a protocol of advantages that make them an indispensable part of many scientific, industrial, and medical practices. However, their extensive use is not an easy ride. Below we will explain in detail the advantages of employing ULT freezers. Although ULT freezers have many advantages, they also relate to problems that must be considered during their selection and use.

One of the best advantages of ULT freezers is that they are capable of delivering safe and long-term storage of sensitive materials [27,119,124,151]. ULT freezers provide temperatures typically between -86°C and -40°C, which are required to avoid biological change and combat any chemical reaction that can degrade the potency of stored materials [5,19,124,151,152]. The relevance of such effective preservation is justified by the growing need to manage delicate biological products, such as vaccines, genetic material, and biologic drugs, which require specific storage requirements to maintain their quality [19,27,28,124,151–153]. ULT freezers protect these items from thermal deviations that would cause loss of integrity [19,27,28,124,151–153].

ULT freezers ensure a rigid and constant temperature, which is extremely crucial for product quality and safety [5,124,152]. Stability at temperature preserves drugs and samples under optimal conditions to prevent the danger of thermal oscillations that can lead to failure or degradation of the product [5,19,28,124,151,152]. Continuous temperature monitoring using in-built monitoring systems ensures that any change is detected instantly, allowing users to take action accordingly [19,52,152,154]. This feature reduces the danger and risk associated with possessing sensitive materials, especially in biomedical clinics and laboratories whose success rests on the integrity of their samples [4,124,144,151].

Temperature recording and monitoring in ULT freezers can be useful in providing product safety and quality data [5,47,124,151]. This capacity enables systematic research into storage conditions and modifications to the management process [5,19,50,124,151]. Such data can be determinative to make conclusions about product quality throughout storage and to make sustained improvement in storage processes [5,19,50,124,151]. In addition, the ability to store information as computerized documents allows for easier analysis and evaluation, thus averting problems potentially caused by human intervention errors [5,19,50,124,151,155]. This characteristic is particularly important where regulatory matters and approving processes are strict, such as in the pharmaceutical sector and biological research [50,124,151,155].

ULT freezers promote research and innovation in reproductive medicine and pharmacology [19,27,28,153]. With their ability for the preservation of fragile samples and biological samples, they help in the process of developing new drugs, vaccines, and biologics [5,19,151]. Maintaining the integrity of samples allows the researchers to perform experiments of high accuracy, and this enhances their ability to detect and verify new drugs of therapy [4,151]. To aid in egg and sperm preservation purposes, ULT freezers play a critical role in supporting procedures that allow the family to achieve their desired outcomes, offering other chances for infertile patients [4,5,19,52,151].

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 May 2025 doi:10.20944/preprints202505.2338.v1

One of the most critical challenges of a ULT freezer is the massive consumption of energy to operate, and this can affect cost as well as the environment [4,19,152]. They store extremely low temperatures for extended amounts of time, and this is what contributes to high electricity consumption [4,5,19,28,151,152]. Increased energy requirements also raise a second question regarding the environmental cost of ULT freezers, as an increasing rate of energy consumption has been linked with CO_2 and other greenhouse gases [19,28,52,152,156]. Therefore, there is considerable need for the development of energy-efficient models and more generally sustainable alternatives that will reduce energy consumption, yet not at the expense of efficiency [19,28,52,152,156].

ULT freezers require periodic maintenance to ensure that they remain in optimal working condition [19,152]. Customers must follow strict maintenance schedules and carry out regular inspections to ensure that refrigeration units operate under normal condition [19,28,52,151]. Any breakdown or default in maintenance can lead to serious consequences, such as the loss of valuable samples and products, which would have a serious impact on research budgets and timelines [19,28,52,151]. The reliability of ULT freezers is also important, considering that malfunctioning in refrigeration can compromise the security of stored materials [19,28,52,151]. That is why the users need to invest in monitoring systems and proactive measures that will ensure timely notification whenever there is any problem [19,151].

One other critical challenge of a ULT freezer is the problems caused by deviations in temperature [27,28,124]. If the temperature is interrupted, even for a short period, serious harm can be caused to stored products [27,28,124]. This situation can be caused by numerous reasons, including mechanical failures, human errors, loss of power supply, or even abrupt climatic upsets [27,28,124]. The damage caused by thermal fluctuation is typically irreparable for sensitive biological materials, such as tissues, vaccines, biological samples, or medications [4,27,28,124]. This means that loss of efficacy or degradation can have far-reaching consequences, both for patients and researchers [4,27,28,124,152]. Preparation degradation has the ability to lead to failures in experiments as well as cause losses of time and money [4,27,28,124]. This is why real-time temperature monitoring systems have to be created, and warning mechanisms are put in place [4,27,28,124,157,158]. The users must be capable of immediately responding to any interruption to prevent damage to [4,27,28,124]. This is a key role in maintaining the integrity of the hundreds or even thousands of samples that can be stored in an ULT freezer [4,27,28,124,157,158].

The future challenge for ULT freezers will be to invest in their sustainability and longevity, to meet new demands and ever-evolving scientific demands [28,152]. The producers need to be constantly on the lookout for innovation and embrace new materials and technologies that will make these freezers compete not only with efficiency but also with energy efficiency [28,152,159–161]. Development of alternative strategies and efficient utilization of energy sources will be crucial to achieving top performance without paying too much [151,152,162,163]. Furthermore, manufacturers will also be required to partner with users in a quest to understand their needs in a bid to develop and design freezers that will keep up with the needs of today's healthcare industry [5,151,152,163].

ULT freezers are an essential piece of equipment in modern scientific and industrial use, with significant advantages that improve the storage and handling of sensitive materials [4,124,152]. Their ability to offer secure and long-term storage, together with offering consistent temperatures, form the basis of many of the important processes in research and science [4,19,27,28,124,152]. Improving the quality of data and promoting innovation makes ULT freezers essential to scientific progress [4,27,28,152].

On the other hand, energy consumption issues, maintenance, and the need to maintain constant temperatures need to be faced and managed [4,19,27,28,124,152]. The users must be informed and trained about maintenance requirements and the potential effects of temperature disturbances [4,19,27,28]. With the present and future needs that are present in science, medicine, and industry, ULT freezers will need to adapt on a continuous basis [4,19,27,28,124,152]. Their advisement towards energy efficiency and greater reliability will be pivotal in making them valuable and sustainable in the long run [4,19,152].

 doi:10.20944/preprints202505.2338.v1

6. Advanced ULT Freezer Technologies

Refrigeration technology has progressed significantly in recent years, offering a variety of systems with different capabilities and features to meet the specialized needs of industry and science. Three prominent systems in refrigeration technology have been chosen to be researched and analyzed to this study and are the Hermetic Compressor Cooling System, the Free Piston Engine, and the Multicompressor Cooling System, however, for confidentiality reasons, no mention will be made of the names of the manufacturers.

Initially, the *Hermetic Compressor Cooling System* uses hermetic compressors arranged in a vertical arrangement [164]. Hermetic compressors, fully closed, prevent liquid leakage, which makes this system ideal for maintaining high efficiency and reliability over time. The vertical arrangement is usually preferable in applications that require rapid temperature reduction or extremely low temperatures, as it allows for greater control and performance. Also, the presence of a Miniature Circuit Breaker (MCB) identifier for protection ensures safety against electrical overloads, enhancing safety and operational reliability. The pressure gauge for monitoring condensation pressure (MR) helps diagnose system performance and ensures optimal operating conditions [164]. This cooling system is suitable for applications where precise temperature control is required, such as in scientific or industrial environments where environmental stability is crucial [164].

On the other hand, the *Free Piston Machine* uses technology that exploits helium as a working fluid [165]. These machines are known for their high efficiency and their ability to provide continuous temperature modulation, cycling on and off, which offers precise and smooth temperature control. Helium, as a working fluid, it is efficient in heat transfer and allows operation at very low temperatures [15]. Continuous configuration capabilities allow the system to quickly adapt to changes in demand or thermal load, while maintaining constant temperatures [165]. This feature makes the Free Piston engine particularly useful in settings where temperature fluctuations can critically affect processes or stored materials. It is ideal for environments that require stable low-temperature conditions with minimal energy consumption and noise, such as medical or laboratory applications [165].

Finally, the *Multi-Compressor Cooling System* has three different compressors, two for the high-temperature system using R134a refrigerant and one for the low-temperature system using R-23 refrigerant. This dual-system installation offers flexible temperature management in a wide range of conditions [166]. Using R134a for high-temperature modes and R-23 for low-temperature modes, the system is capable of effectively handling cooling variations [166]. The multicompressor design optimizes performance in different temperature zones, enabling precision and efficiency in complex refrigeration applications [166]. This versatile system is particularly suitable for industrial applications, where different parts may require different temperature settings or where deep-freezing capabilities are needed [166].

ULT cooling technologies have advanced quickly, bringing with them a wide range of systems tailored to different industrial, preservation, and research requirements. The strengths and applicability of three well-known systems—the multi-compressor systems, the hermetic compressor, and the free-piston engine—are compared and examined below. Regarding cooling performance, energy efficiency, environmental impact, safety features, and connectivity, each system exhibits distinct capabilities that represent various priorities in various operating environments. To guarantee the integrity, sustainability, and safety of priceless biological samples and delicate materials, this analysis attempts to give a thorough overview that will direct the choice of the best cooling solution, customized to the unique needs of research institutions, industrial facilities, and transportation logistics as applicable.

All three systems offer excellent cooling performance, with the free-piston engine being recognized for its widest temperature range, giving it an advantage in storing various biological samples [164–166]. The hermetic compressor offers reliability through its sealed cooling system, as well as excellent thermal insulation, while the compressor system ensures reliable cooling with its dual system and its ability to operate in extreme conditions. Moreover, energy efficiency is critical for sustainability [164–166]. The free-piston engine consumes significantly less energy compared to traditional units and offers up to 40% energy savings. The hermetic compressor also has energy-saving programs, but it does not have the same scale as the free-piston engine [164–166]. The multicompressor system, although it incorporates a dual system, also requires maintenance that can affect efficiency.

The hermetic compressor control system is highly advanced, with remote monitoring and data management capabilities, however, the free piston machine similarly offers excellent temperature recording and monitoring through its parish interfaces. The multi-compressor system focuses more on cooling integrity in extreme environments and less on advanced management features [164–166]. Moreover, all systems either use natural refrigerants or contain features that reduce the environmental footprint [164–166]. The free piston engine excels for its extremely low energy consumption and the use of environmentally friendly refrigerants, while the hermetic compressor offers energy programs and advanced insulation processes. The multi-compressor system uses refrigerants with minimal potential to deplete the ozone of the environment [164–166].

Safety is paramount for ULT systems, as minimizing risks is imperative when storing valuable biological samples [164–166]. The hermetic compressor has safety thermostats and optional voltage stabilizers, protecting the liner from voltage fluctuations. Correspondingly, the free-piston machine includes advanced security features, providing locks and PINs to access the GUI, ensuring that only authorized personnel can make changes [164–166]. The multi-compressor system also includes safety features through data control and temperature monitoring, reducing the possibility of load tampering [164–166].

Supporting functions are critical for decision-making in modern research facilities. All three systems offer advanced capabilities, but with a different focused impact. The hermetic compressor provides the most sophisticated connectivity options, facilitating integration with laboratory networks and enabling real-time monitoring. The free piston machine offers applications for monitoring in remote locations, with 40% energy savings. The multi-compressor system, although less focused on connectivity, offers excellent flexibility during transport and storage, with the possibility of automatic reverse operation containing safety features to protect the load.

At a time when research and science require strict storage standards, the three ULT systems analyzed below represent market-leading choices. In summary, each option seems suitable for different working conditions and user conditions [164–166]. The final selection should be based on the specific needs of the research institutions, including external actors, infrastructure and available resources, to ensure the integrity and sustainability of the stored samples. Each cooling system offers unique advantages that are tailored to specific applications [164–166]. The Hermetic Compressor Cooling System is optimal for environments that require low temperatures and increased control, ranks as the leader in innovative features, durability and energy efficiency [164–166]. It offers advanced connectivity features that make it ideal for laboratory environments that need accurate data monitoring and management [164–166]. The Free Piston engine excels in settings that require energy efficiency with continuous operation, can be an important choice for laboratory requirements with strict temperature requirements. Free piston engine technology guarantees high reliability with minimal maintenance [164–166]. The Multi Compressor Cooling System provides adaptive cooling solutions that meet a variety of temperature requirements. It aims to protect and transport sensitive products at extreme temperatures. The dual cooler system ensures continuous cooling and flexibility in the transport sector [164–166].

These technologies provide reliable and effective solutions, making a significant contribution to the advancement of science and industrial production, as well as to the protection of sensitive materials that require special storage conditions. The following Table 3 shows a comparative analysis of low temperature cooling systems as derived from our market knowledge.

doi:10.20944/preprints202505.2338.v1

Table 2. Comparative Analysis of Low Temperature Cooling Systems.

Features	Hermetic Compressor	Free Piston Engine	Multi-compressors
References	164-166	164-166	164-166
Capacity	706 liters	780 liters	59,720 liters
Temperature Range	-40°C to -85°C	-20°C to -86°C	0°C to -60°C
Construction Material	AISI 304 Stainless Steel	Blank insulation panels	Special design with two systems
Cooling Technology	Hermetic compressors	Free-piston Stirling	Dual cooling system
Energy Consumption	Energy Saving Strategies	~6.67 kWh/d, up to 40% less energy	Requires maintenance for stable operation
Connectivity	USB, SIM, Wi-Fi, Ethernet	Remote Monitoring	Limited options
Temperature Stability	Superior thermal performance	± 1 °C	Constant temperature control
Operating Safety	Safety thermostats	Lock and PIN for access	Diagnostics and Warnings
Maintenance Procedures	Regular programs necessary	Maintenance with GUI and automated monitoring	Regular maintenance and checks with automated diagnostics
Useful Life	10-12 years	12 years	10-12 years
Refrigerant Safety	HCFC or CFC free R-170 and R-1270	Uses R-170 (Ethane), eco-friendly	Uses HFCs, requires caution due to flammability
Operating Noise	Noise during operation	<48 dB(A)	Noise during operation
Environmental policy	Eco-friendly refrigerants	Uses natural refrigerants	Low ozone depletion potential
Resistance to Temperature Fluctuations	High	High	High via dual compressors

Features	Hermetic Compressor	Free Piston Engine	Multi-compressors
References	164-166	164-166	164-166
Reliability Price	High reliability	Variable operation with minimal maintenance	Excellent due to redundancy
Measurement	1990x1060x1000 mm	1994x870x915 mm	10945x2154x2896 mm

Hermetic compressors, Free Piston Machine and multi compressor freezers are shown in the following figures (Fig. 1 and 2).

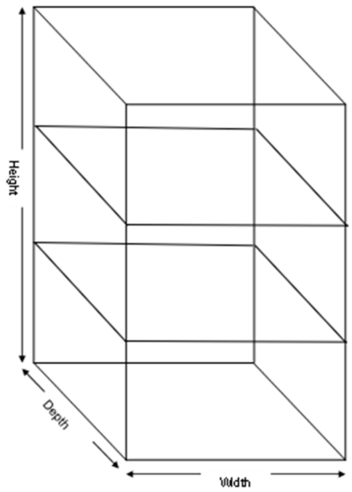

Figure 1. Hermetic Compressor and Free Piston Machine.

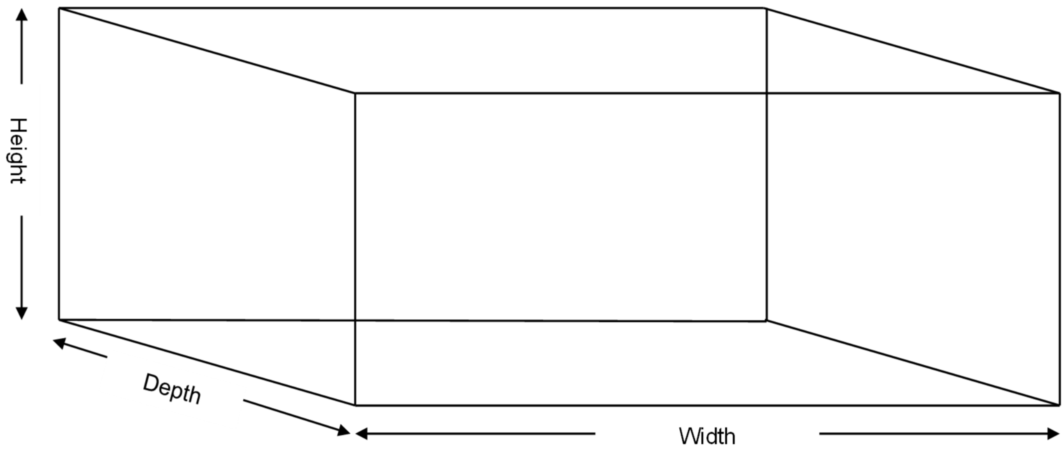

Figure 2. Multi-compressors.

7. Regulatory Compliance

Evaluation of temperature uniformity and efficiency of refrigerating equipment is one of the core elements of quality assurance for many industries, including pharmaceutical, biological, and food industries [21,22]. Equipment for refrigeration, especially at extremely low temperatures, must be put through strict validation procedures in order to verify that it is in accordance with regulatory requirements and specifications for perishable storage [167,168]. National and international policymaking can be a significant contributor to making the samples safe and propelling innovation in ULT freezers [21,22]. Regulators are required to communicate with industries and research organizations to establish regulations which will make the freezers qualify against stringent performance and temperature criteria [21,22]. Furthermore, financing the grant of money and research schemes is likely to catalyze studies into fresh and improved cold storage technology and consequently enhance innovative storage solution developments and deployment [21,22,167,168]. A summary overview of the regulation is presented at Table 4 below.

Management of operations and facilities for many industries is paramount to the efficiency and regulatory compliance of processes in line with regulatory standards [21,22,167,168]. Hence, storage of critical products and samples is necessary in various industries like biomedical research, healthcare, food storage, and manufacturing [21,22,167,168]. Functioning of ULT freezers plays a critical role in maintaining the integrity and lifespan of such products that must be kept constantly exposed to ultra-low temperatures in order to prevent deterioration and make them sustainable [21,22,167,168]. During ULT freezer testing, some key performance characteristics must be tested, such as temperature stability, temperature uniformity, peak fluctuation, recovery time, energy efficiency, operational noise, and general durability and reliability [21,22,167,168].

Temperature stability and uniformity are two critical elements for evaluation of the performance. Temperature stability is the ability of an ULT freezer to retain a stable temperature over time with minor temperature variations [21,22,167,168]. This stability is necessary to prevent compromising sensitive products. In the pharmaceutical industry, for example, temperature instability can be detrimental to vaccines and biologics, rendering them ineffective [160,167,168]. High-quality ULT freezers must be designed to provide even low temperatures and therefore maintain a wide range of sensitive materials from temperature-induced degradation [21,22,167,168]. On the other hand, temperature uniformity ensures that the entire storage space within the ULT freezer remains at a constant temperature [21,22,86,167,168]. Effective insulation and well-designed air flow networks help to eliminate hot and cold spots inside the freezer’s chamber so that products can be stored anywhere without risk [19,152]. This is especially important in larger freezers, where sudden temperature gradients can be present from the center outward [19,152]. Impeccable temperature consistency must be ensured to provide equal environmental conditions to all samples and products, keeping the chances of local degradation to a bare minimum [19,152].

Peak variance is an essential performance metric that captures the largest deviation from the set temperature at any moment, including those infrequent but temporary fluctuations [19,20]. An ULT freezer is of highest priority to those industries that have extremely sensitive materials, such as pharmaceutical and biotechnology industries, where slight variations in temperature can lead to reduced efficiency or alteration [19,20]. Laboratories whose equipment has to be validated with utmost accuracy are careful to install ULT freezers with stringent specifications to ensure maximum retention of their precious supplies [19,20].

Door opening recovery time is an important measurement, which determines the degree to which a freezer can recover to the specified temperature after opening [21,22,167,168]. Short recovery times are critical in applications involving fast access, such as research laboratories and pharmaceutical storage facilities [21,22,167,168]. Limiting exposure of sensitive materials to warmer air when opening doors prevents defrosting and degradation [21,22,167,168]. ULT freezers with rapid recovery facilities are particularly worth it in high-efficiency settings [21,22,167,168].

Energy efficiency is increasingly important because of the high energy costs associated with operating a series of ULT freezers [51,162]. Use of energy-efficient models can reduce operation costs and support organizational sustainability efforts [52,162]. ULT freezers that use natural refrigerants and variable speed compressors can optimize cooling efficiency with less energy consumed [52]. The reverse is the case when it comes to older units that utilize conventional refrigerants and constant-speed compressors, which consume more energy and cost [52].

The performance and long-term reliability of ULT freezers are dependent on durability and the quality of construction [28,51,52]. The freezers must be built with high-grade materials that resist the toughness of continuous use [28,51,52]. Durable external frames and internally supported

 doi:10.20944/preprints202505.2338.v1

components guarantee the life of the units while cutting the risk of breakdowns [28,51,52]. Advanced alarm and monitoring systems that provide real-time performance data are also significant features, allowing users to easily address any issue that may undermine the integrity of their products stored [28,51,52].

ULT freezers are precious resources within many industries, particularly useful for the preservation of sensitive materials [22,160]. The freezers operate below temperatures much colder than -70°C, allowing product integrity to be maintained, which otherwise rapidly degrades [21,22,136,169]. In view of the extreme importance of freezers in ULT, regulatory bodies such as the European Union (EU) and United States Food and Drug Administration (FDA) have formulated comprehensive guidelines so that freezers in ULT are in line with stringent safety, quality, and efficacy standards [170,171]. This essay examines such regulatory contexts, the time-consuming process to confirm ULT freezers, and provides some insights into how organizations apply such guidelines in day-to-day practice for making the world safe [170–172].

The European Union, as a result of its extensive regulatory standards, plays a significant role in deciding how materials are stored, like the ones that require ULT conditions. Firstly, Volume 4 of EudraLex clarifies the Good Manufacturing Practice (GMP) regulations regarding the production and storage of medicinal products [170]. These standards emphasize controlled storage conditions and arrange equipment such as ULT freezers to be certified so that they meet the required parameters [170]. GMP guidelines require stringent assessment of storage conditions so that there is no compromise on product quality [170]. Moreover, the GDP Guidelines (2015/C 95/01) focus on the supply chain dimension, highlighting that both storage and transport maintain product integrity from manufacturing through to delivery [171]. For ULT freezers, this means that the end-to-end supply chain can hold ultra-low temperatures without any deviation, avoiding potential spoilage of temperature-sensitive medicines [173]. Continuous monitoring and validation processes are required by these guidelines to maintain compliance [173]. In addition, Directive 2000/54/EC makes reference to the safety of workers against the risks from exposure to biological agents, which can involve handling and storage in ULT freezers [173]. It demands adequate safety measures such that any staff members who work with these freezers are sufficiently trained and equipped [170,172–176]. Meanwhile, the Directive 2009/41/EC legislates the use of genetically modified organisms (GMOs) with degrees of restriction in tandem with safe measures for storage, once again necessitating precise control of temperature and further facilitated through ULT freezers [173]. Finally, the European Medicines Agency (EMA) gives scientific advice which complements these guidelines by focusing on the quality of biological drugs stored under ULT conditions [170,172–176]. Although not enforceable by law, these recommendations have an impact on national requirements in all EU Member States and ensure a harmonized approach towards the validation of ULT storage [170,172–176].

The United States has a strong regulation by the FDA that secures storage requirements of products. Title 21 CFR Part 11 plays a key role in governing electronic records and signatures, data integrity, and traceability of temperature records of ULT freezers [171,177]. This section demands the inflexibility of use of electronic monitoring systems with assurances that all data are secure and reliable. Section 210 and 211 have cGMP regulations for drugs, demanding storage conditions, such as equipment like ULT freezers, to have strict operational requirements [171,177]. This ensures consistent production and storage conditions, ensuring product efficacy and patient safety [171,177]. The FDA's Biotechnology Inspection Guide offers supporting materials and reports required to comprehend the intricacies of organic storage [171,177].

Apart from regulatory requirements, several industry standards offer additional guidance, guaranteeing holistic best practices in the validation of ULT freezers. Standards for (bio)processing equipment have been defined by the American Society of Mechanical Engineers (ASME) BPE-2014, which also affect the validation and design of ULT freezers [158]. These standards allow for an apparatus to ensure the performance and reliability of the equipment at extremely low temperatures. The Pharmaceutical Inspection Co-operation Program (PIC/S) has globally acknowledged recommendations (PE-009-9 and PE-009-11) to ensure Member States, storage sites, and manufacturing areas implement best-quality procedures [158].

The International Society of Pharmaceutical Engineering (ISPE) publishes significant guidelines, such as the Baseline Guide for Active Pharmaceutical Ingredients and the Good Practice Guide for Cold Chain Management, which reflect industry best practices for validating cold control systems in ULT freezers [21,22].These reports emphasize the importance of chamber monitoring and mapping, promoting a scientific method to achieving temperature consistency in each storage chamber [21,22].

The European Commission revised the Good Distribution Practices (GDP) guidelines on 5 November 2013 (PIC/S), considering more advanced supply chains [170]. The storage needs of drugs must be complied with when stored and transported in the specified limits as proposed by manufacturers or on external packaging [172]. GDP emphasizes extreme vigilance for the integrity of the supply chain. The same is guaranteed through the preservation of drug quality from manufacturing to final point of storage, distribution and sale, ultimately ending at the end-user or patient [172]. Storage and transport of pharmaceutical and biological material need to be monitored continuously with calibrated systems for continuous verification or qualified transport systems based on historical process data [21,22,172]. This includes the use of monitoring as a tool for continuous verification and validation of the process, along with defining facilities and equipment, as part of standard daily inspection practices [21,22,172]. Apart from this, maintaining precise temperature control during the supply chain is difficult and an essential process in guaranteeing the quality of drugs, especially where precise temperature management matters [21,22,172]. Therefore, certification is a key quality assurance tool for the pharmaceutical sector, certifying that the equipment, facilities and systems are working in the right way and are producing the intended results [21,22,172]. Storage facilities designation is a crucial and most used criterion in worldwide standards [21,22,136,169,172].

It is essential to create national and international policies in order to ensure the security of samples and foster innovation in the creation of ULT freezers [21,22]. As the preservation of sensitive material in different fields must be strongly controlled, it is essential to perform collaborations between regulators, industry, and centers for research in order to guide the implementation of rules which respond to the current needs [21,22,169,172]. In particular, the FDA and the EU are critical in implementing and sustaining regulatory systems that ensure product quality and safety across all processes – storage, distribution, and production [21,22,169,172]. Compliance with the guidelines released by these organizations and application of best practices in the handling of ULT freezers ensure uniformity and integrity of the samples [2,21,169,172]. Moreover, research grants funding and supporting new refrigeration technologies lead to continuous improvement in storage systems, which is crucial for the development of medicine and science [21,22,136,169,172].

In conclusion, the performance of the ultra-low temperature freezer is crucial for secure product storage of a broad scope of products across numerous industries [28,51,52]. Temperature stability, uniformity, peak fluctuation, recovery time from door opening, energy efficiency, operating noise, and durability are all matters of concern to watch out for when choosing an ULT freezer [51,52]. Through prioritizing such performance measures, organizations maintain the integrity of their critical samples and products and ultimately maintain operational success and protect public health [51,52]. Collectively, the teamwork of the regulators and industry stakeholders and the support of research are priceless in developing and implementing effective and secure storage solutions [21,22,167,168]. By reviewing regulatory processes and conformity, the call for the need to constantly educate and remain aware in controlled storage is strengthened, thereby a safer and more effective future in many different fields [21,22,167,168].

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 May 2025 | doi:10.20944/preprints202505.2338.v1

Table 4. Regulatory requirements.

Source	Title of the Regulation	Article	References
EU	Eudralex; Volume 4 GMP Guidelines	Volume 4	170
	Good Distribution Practice of active substances for medicinal products for human use (2015/C 95/01)	2015/C 95/01	172
	Directive 2000/54/EC Protection of Workers from Risks Related to Exposure to Biologic Agents at Work	2000/54/EC Annex V&VI	173
	Directive 2009/41/EC on the contained use of genetically modified micro-organisms	2009/41/EC	175
	European Medicines Agency scientific guidance documents on biological drug substances	N/A	174
US FDA	Title 21 Code of Federal Regulations, Electronic Records & Electronic Signatures	Part 11	171
	Title 21 Code of Federal Regulations, Current Good Manufacturing Practice, Processing, Packing or Holding of Drugs; General	Part 210	177
	Title 21 Code of Federal Regulations, Current Good Manufacturing Practice for Finished Pharmaceuticals	Part 211	177
ASME	Bioprocessing Equipment	ASME BPE-2014	158
PIC/S	Guide to Good Manufacturing Practice for Medicinal Products, Part I	PE-009-9	158
	Guide to Good Manufacturing Practice for Medicinal Products, Part II	PE-009-11	158
ISPE	Baseline Guide: Biopharmaceuticals	Volume 6. 2nd	21,22
	Baseline Guide: Commissioning and Qualification	Volume 5, 2nd	21,22
	Good Practice Guide -Cold Chain Management	2011	21,22
	Good Practice Guide – Controlled Temperature Chamber Mapping and Monitoring	2016	21,22

8. Conclusions

With all the above in mind, the history of ULT freezers has been significantly shaped by advances in refrigeration technology. There has been a transition, over time, from traditional vapor compression systems to more complicated multi-stage refrigeration systems [178]. Advances in materials and engineering have seen freezers featuring a low temperature range of as low as -86°C. Microprocessor integration applied in monitoring and temperature control has increased the reliability of operation, and better insulating materials, such as polyurethane, have improved the efficiency of energy. All these developments allow ULT freezers to provide constant temperatures, which is vital in the protection of sensitive biological materials and supporting state-of-the-art research in various fields [178].

Different models of refrigerants condition the effectiveness and eco-friendliness of ULT freezers. Previously, CFCs were widely used but phased out due to their destructive impact on the ozone layer. HFCs were a temporary solution, giving excellent thermal performance but high GWP problems. The emphasis on sustainability today has led to the quest for replacements such as HFOs and natural refrigerants such as ammonia and carbon dioxide, with low GWP values. The selection of an appropriate refrigerant is important, as the same can influence directly both the performance of the freezer in terms of cooling as well as its environmental impact, leading the way towards more eco-friendly refrigeration technology [178].

The advantages of ULT freezers are tremendous, particularly in applications critical to life such as healthcare and scientific studies. Their ability to store sensitive materials like vaccines, biological specimens, and genetic material under secure, controlled and long-term conditions preserves their integrity and functionality [178]. Further, ULT freezers enable scientists to plan experiments and trials with exact accuracy, enabling enhancement in biotechnology, pharmacology, and reproductive medicine [178]. But there are also problems, such as high energy consumption and maintenance, which can lead to operational costs and certain risks from temperature fluctuations. Problems must be aggressively monitored and proactively managed methods to overcome risk and achieve the best performance [178].

ULT freezer compliance specifications are needed to ensure safe storage of sensitive materials to a wide variety of industries. Regulatory agencies, like FDA and EMA, have promulgated good guidelines to manage the operation of such freezers, including checking equipment certification as well as stern validation protocols. In ensuring an efficient long lifespan along with the competence of ULT freezers, as per regulatory policies, stakeholders must spend on certain strategies and good practices [178]. Comprehensive and on-going training of staff in operation, maintenance, and importance of temperature control are essential in quality control maintenance [178]. Application of sophisticated monitoring systems that issue real-time warnings makes it easier to quickly identify and correct any temperature deviations or mechanical failures, thereby protecting valuable samples. Users and manufacturers also must collaborate to adopt energy-saving technologies like improved refrigerants and enhanced insulation [178]. By prioritizing routine maintenance and having well-defined procedures, stakeholders will be able to reap the maximum amount of operating time and efficiency from ULT freezers, maintaining compliance with industry standards and protecting public confidence in stored biological material [178].

Environmental concerns play an increasing role in the choice of cooling technologies. HFC-based refrigerants used in hermetic and multi-compression systems are being phased out due to the high global warming potential [54,57]. The free-piston engine offers a viable alternative by allowing the use of natural refrigerants such as helium and nitrogen, which align with current regulatory trends favoring low GWP solutions [53,55]. However, despite their environmental benefits, free piston engines require specialized maintenance and know-how, limiting their widespread adoption in commercial ULT applications [35]. Multi-compressor systems, while still relying on conventional refrigerants, can be optimized with alternative low-GWP refrigerants, such as HFOs or CO_2 to reduce environmental impact [60,92].

 doi:10.20944/preprints202505.2338.v1

The study's findings provide useful insights into industries that rely on ULT freezers, including biomedical research, pharmaceuticals, and biotechnology [176]. The superior temperature uniformity of multi-compressor systems makes them ideal for large-scale bio-storage and clinical specimen storage. However, higher energy consumption requires strong energy management strategies to compensate for operating costs [27]. Free-piston engines, with their energy efficiency and environmental advantages, are suitable for applications where sustainability and long-term cost savings are prioritized. However, their slow recovery times limit their suitability for applications that require frequent access or high thermal resistance [4]. Hermetic compressors, despite their lower efficiency, remain viable for budget-conscious applications where moderate temperature fluctuations are acceptable [44].

The future of ultra-low temperature freezers is extremely bright, not only for supporting research and medicine but also for supporting environmentally friendly practice [178]. The future growth of these technologies will be influenced by cross-disciplinary cooperative work among industry professionals, scientists, and regulatory bodies to ensure that ULT freezers are maintained as central tools of today's scientific exploration [178]. By their focus on the invention and application of effective refrigeration technologies, the industry will work toward fostering a culture of accountability, safety, and innovation that will result in innovations capable of improving public health as well as advancing the cause of many different sciences [178].

Future research should investigate long-term operational stability, maintenance costs, and life-cycle environmental impacts in different refrigeration systems [50]. In addition, advances in alternative refrigerants and hybrid refrigeration technologies could provide innovative solutions to balance energy efficiency, environmental sustainability and reliability of performance [62]. Future studies should explore the integration of phase change materials (PCM) and AI-driven adaptive cooling control to enhance temperature stability while minimizing energy consumption [48]. Studies could delve into longitudinal analyses of ULT freezer performance over extended periods, looking at how advances in technology affect operational efficiency. In addition, investigating user behaviors regarding freezer use and maintenance practices can yield actionable insights that align operational practices with technological capabilities. In addition, expanding this research to include a wider variety of environments and use cases can help identify industry-wide best practices that offer significant energy savings while ensuring product safety and service life. Researchers can also consider the possibility of integrating smart technology into freezer systems to provide real-time monitoring, predictive maintenance alerts, and automated operational adjustments based on environmental conditions [178]. Exploring the potential benefits of demand response strategies to freezer operations could yield insights into how to manage energy consumption during peak hours while maintaining efficiency [154]. Combining these strategies with evolving machine learning technologies can inform user-generated practices that lead to improved user experience and operational efficiency [178].

In conclusion, this review recognizes the significant advancement of ULT freezers and their inherent use in preserving sensitive materials and facilitating critical research in biotechnology and medicine. The analysis of other forms of refrigerants proves the trend in the future to be ecologically friendly decisions, while conformity requirements analysis and testing procedures identify the need to honor stringent safety controls. The review also describes the advantages and disadvantages of installing and operating ULT freezers, providing great insights into best practice and management. In addressing these topics, this review contributes to the development of the subject area through the provision of a comprehensive understanding of the technological and regulatory context of ULT freezers. However, note that there are areas of opportunity, notably in energy efficiency and the use of new monitoring technologies that are ripe for additional research. Continued study in these areas not only stands to improve the effectiveness and sustainability of ULT freezers but also will keep them at the forefront as tools for driving scientific progress and improving public health outcomes.

Author Contributions: All authors have written, reviewed and edited, read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Data Availability Statement: no new data were created.

Acknowledgments: The authors have reviewed and edited the output and take full responsibility for the content of this publication

Conflicts of Interest: The authors declare no conflicts of interest

References

1. Kamenski, G., Ayazseven, S., Berndt, A., & et al. (2020). Clinical relevance of CYP2D6 polymorphisms in patients of an Austrian medical practice: A family practice-based observational study. Drugs - Real World Outcomes, 7, 63–73. https://doi.org/10.1007/s40801-019-00177-4
2. Middelburg, R. A., Borkent, B., Jansen, M., van de Watering, L. M. G., Wiersum-Osselton, J. C., Schipperus, M. R., Beckers, E. A. M., & Briët, E. (2011). Storage time of blood products and transfusion-related acute lung injury. Transfusion. https://doi.org/10.1111/j.1537-2995.2011.03352.x
3. Sun, J., Zhang, M., Gehl, A., Fricke, B., Nawaz, K., Gluesenkamp, K., Shen, B., Munk, J., Hagerman, J., & Lapsa, M. (2022). COVID-19 vaccine distribution solution to the last mile challenge: Experimental and simulation studies of ultra-low temperature refrigeration system. International Journal of Refrigeration, 133, 313-325. https://doi.org/10.1016/j.ijrefrig.2021.11.005
4. Xu, Q., Deng, B., Wang, Y., Liu, W., & Chen, G. (2023). Small, affordable, ultra-low-temperature vapor-compression and thermoelectric hybrid freezer for clinical applications. Cell Reports Physical Science, 4(101735). https://doi.org/10.1016/j.xcrp.2023.101735
5. Powell, S., Molinolo, A., Masmila, E., & Kaushal, S. (2019). Real-Time Temperature Mapping in Ultra-Low Freezers as a Standard Quality Assessment. Biopreservation and biobanking, 17(2), 139–142. https://doi.org/10.1089/bio.2018.0108
6. Ahamed, J. U., Saidur, R., & Masjuki, H. H. (2011). A review on exergy analysis of vapor compression refrigeration system. Renewable and Sustainable Energy Reviews, 15(3), 1593-1600. https://doi.org/10.1016/j.rser.2010.11.039
7. Barbosa, J. R., Ribeiro, G. B., & de Oliveira, P. A. (2011). A State-of-the-Art Review of Compact Vapor Compression Refrigeration Systems and Their Applications. Heat Transfer Engineering, 33(4–5), 356–374. https://doi.org/10.1080/01457632.2012.613275
8. PHcbi (2024). Choosing a reliable ULT freezer: What you need to know. Retrieved from: https://www.phchd.com/us/biomedical/blog/choosing-a-reliable-ult-freezer-what-you-need-to-know?mtm_source=google&mtm_medium=cpc&mtm_campaign=21676150519&mtm_cid=21676150519&mtm_kwd=&mtm_content=&gad_source=1&gclid=Cj0KCQiAgdC6BhCgARIsAPWNWH3HWD6O4q4oY7PaoZsPUYSWoxC7salpyCivuOlz51WkYJ86su5nsRAaAgMxEALw_wcB [Accessed on 07DEC2024]
9. Zhao, H., Hou, Y., & Chen, L. (2009). Experimental study on a small Brayton air refrigerator under −120°C. Applied Thermal Engineering, 29(8–9), 1702–1706. https://doi.org/10.1016/j.applthermaleng.2008.07.028
10. Gondrand, C., Durand, F., Delcayre, F., Crispel, S., & Gistau Baguer, G. M. (2014). Overview of Air Liquide refrigeration systems between 1.8 K and 200 K. AIP Conference Proceedings, 1573(1), 949–956. https://doi.org/10.1063/1.4860807
11. Pan, M., Zhao, H., Liang, D., Zhu, Y., Liang, Y., & Bao, G. (2020). A review of the cascade refrigeration system. Energies, 13(9), 2254. https://doi.org/10.3390/en13092254
12. Saravacos, G., & Kostaropoulos, A. E. (2016). Refrigeration and freezing equipment. In Handbook of food processing equipment (Food Engineering Series). Springer. https://doi.org/10.1007/978-3-319-25020-5_9
13. Arshad, M. O., Azam, Q., Ahmad, S. T. I., Khan, F., & Wahid, M. A. (2021). Analysis of vapour compression refrigeration system with R-12, R-134a and R-22: An exergy approach. Materials Today: Proceedings, 46(Part 15), 6748-6752. https://doi.org/10.1016/j.matpr.2021.04.278

14. Mota-Babiloni, A., Mastani Joybari, M., Navarro-Esbrí, J., Mateu-Royo, C., Barragán-Cervera, Á., Amat-Albuixech, M., & Molés, F. (2020). Ultralow-temperature refrigeration systems: Configurations and refrigerants to reduce the environmental impact. International Journal of Refrigeration, 111, 147–158. https://doi.org/10.1016/j.ijrefrig.2019.11.016
15. Wen, M.-Y., Ho, C.-Y., & Hsieh, J.-M. (2006). Condensation heat transfer and pressure drop characteristics of R-290 (propane), R-600 (butane), and a mixture of R-290/R-600 in the serpentine small-tube bank. Applied Thermal Engineering, 26(16), 2045-2053. https://doi.org/10.1016/j.applthermaleng.2005.10.001
16. Lim, J.Y., Kim, N.A., Lim, D.G. et al. Process cycle development of freeze drying for therapeutic proteins with stability evaluation. Journal of Pharmaceutical Investigation 46, 519–536 (2016). https://doi.org/10.1007/s40005-016-0275-7
17. Ye, W., Yan, Y., Zhou, Z., & Yang, P. (2024). Parametric analysis and performance prediction of an ultra-low temperature cascade refrigeration freezer based on an artificial neural network. Case Studies in Thermal Engineering, 55, 104162. https://doi.org/10.1016/j.csite.2024.104162
18. Berchowitz, D., & Kwon, Y. (2012). Environmental profiles of stirling-cooled and cascade-cooled ultra-low temperature freezers. Sustainability, 4(11), 2838–2851.
19. Saeed, M. Z., Contiero, L., Blust, S., Allouche, Y., Hafner, A., & Eikevik, T. M. (2023). Ultra-low-temperature refrigeration systems: A review and performance comparison of refrigerants and configurations. Energies, 16(21), 7274. https://doi.org/10.3390/en16217274
20. Tan, H., Xu, L., Yang, L., Bai, M., & Liu, Z. (2022). Operation performance of an ultralow temperature cascade refrigeration freezer with environmentally friendly refrigerants R290-R170. University of Electronic Science and Technology of China Zhongshan Institute. https://doi.org/10.21203/rs.3.rs-2004580/v1
21. ISPE. (2016). Good practice guide: Controlled temperature chamber mapping and monitoring. International Society for Pharmaceutical Engineering.
22. ISPE. (2019). Commissioning and Qualification. Volume 5, 2nd Edition. International Society for Pharmaceutical Engineering.
23. Moerman, F., & Fikiin, K. (2016). Hygienic design of air-blast freezing systems. In Handbook of hygiene control in the food industry (2nd ed., pp. 271-316). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100155-4.00020-0
24. Meir, K., Cohen, Y., Mee, B., & Gafney, E. (2014). Biobank networking for dissemination of data and resources: An overview. J Bioreposit Sci Appl Med, 2, 29.
25. Paradiso, A. V., Daidone, M. G., Canzonieri, V., & Zito, A. (2018). Biobanks and scientists: Supply and demand. Journal of Translational Medicine, 16(1), 136. https://doi.org/10.1186/s12967-018-1505-8
26. Scudellari, M. (2013). Biobank managers bemoan underuse of collected samples. Nature Medicine, 19(3), 253–263.
27. Dhiman, P., & Kumar, A. (2023). A situational based reliability indices estimation of ULT freezer using preventive maintenance under fuzzy environment. International Journal of Mathematical Engineering and Management Sciences. https://doi.org/10.33889/IJMEMS.2023.8.3.0xx
28. Farley, M., McTier, B., Arnott, A., & Evans, A. (2015). Efficient ULT freezer storage. Social Responsibility & Sustainability: University of Edinburgh. Retrieved November 18, 2024 from https://www.ed.ac.uk/files/atoms/files/efficient_ult_freezer_storage.pdf
29. Hajagos, B. (2021). Life cycle assessment and emission reduction of cascade and Stirling ultra-low temperature freezers [Master's thesis, Eindhoven University of Technology]. Retrieved November 18, 2024, from https://pure.tue.nl/ws/portalfiles/portal/183343183/1492306_MasterThesisBenceHajagos.pdf
30. Liu, J., Yu, J., & Yan, G. (2024). Experimental study on Joule-Thomson refrigeration system with R1150/R290/R601a for ultra-low temperature medical freezer. Applied Thermal Engineering. https://doi.org/10.1016/j.applthermaleng.2024.124015
31. Xiao, R., Zhang, Y., Liu, X., & Yuan, Z. (2015). A life-cycle assessment of household refrigerators in China. Journal of Cleaner Production, 15(95), 301–310.

32. Wang, L. Y., Cui, J. J., Liu, J. Y., & et al. (2018). Gene-gene and gene-environment interaction data for platinum-based chemotherapy in non-small cell lung cancer. Scientific Data, 5, Article 180284. https://doi.org/10.1038/sdata.2018.284
33. Authelin, J.-R., Rodrigues, M. A., Tchessalov, S., Singh, S. K., McCoy, T., Wang, S., & Shalaev, E. (2020). Freezing of biologicals revisited: Scale, stability, excipients, and degradation stresses. Journal of Pharmaceutical Sciences, 109(1), 44-61. https://doi.org/10.1016/j.xphs.2019.10.062
34. Sun, J., Zhang, M., Gehl, A., & et al. (2022). Dataset of ultralow temperature refrigeration for COVID-19 vaccine distribution solution. Scientific Data, 9, 67. https://doi.org/10.1038/s41597-022-01167-y
35. Owusu-Apenten, R. & Vieira, E.R. (2023). Chapter 13 Low-Temperature Preservation. In: Book elementary Food Science. Food Science Text Series. Springer, 5th edition, pp. 289-316. DOI: https://doi.org/10.1007/978-3-030-65433-7_13
36. Rees, J. (2013) Refrigeration nation: a history of ice, appliances, and enterprise in America. Retrieved from https://books.google.co.uk/books?id=JeoEAQAAQBAJ
37. Gavroglu, K. (2014). Historiographical Issues in the History of Cold. In: Gavroglu, K. (eds) History of Artificial Cold, Scientific, Technological and Cultural Issues. Boston Studies in the Philosophy and History of Science, vol 299. Springer, Dordrecht. DOI: https://doi.org/10.1007/978-94-007-7199-4_1
38. Robinson, L.M. 1997. Safeguarded by your refrigerator: Mary Engle Pennington's Struggle with the National Association of Ice Industries. In Rethinking home economics: Women and the history of a profession, ed. Sarah Stage and Virginia B. Vincenti, 253–270. Ithaca: Cornell University Press.
39. Pennington ME (1912) The hygienic and economic results of refrigeration in the conservation of poultry and eggs. Am J Public Health 2(11):840–848. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18008742
40. Thermo scientific (2024). Top considerations for selecting a ULT Freezer – application. Retrieved December 3, 2024 from: https://assets.thermofisher.com/TFS-Assets/LPD/Product-Information/Top-Considerations-ULT-Space-Constraints-TNTPCONSPACE-EN.pdf
41. Mallett, C. P. (1993). Frozen food technology. Springer. Retrieved from https://books.google.nl/books?hl=en&lr=&id=rzk9b3NpUzwC&oi=fnd&pg=PA20&dq=+William+Cullen+and+freezing&ots=1fbQor9ixv&sig=AhoQszLuugb-DMaNJxXQiGfpJ8g&redir_esc=y#v=onepage&q=William%20Cullen%20and%20freezing&f=false
42. Prasad, U. S., Mishra, R. S., & Das, R. K. (2024). Study of vapor compression refrigeration system with suspended nanoparticles in the low GWP refrigerant. Environmental Science and Pollution Research, 31, 1–26. https://doi.org/10.1007/s11356-023-30596-4
43. Thevenot, R. (1979). A history of refrigeration throughout the world. Trans. from French J.C. Fidler. Paris: International Institute of Refrigeration.
44. Graham, M., Samuel, G. & Farley, M. Roadmap for low-carbon ultra-low temperature storage in biobanking. J Transl Med 22, 747 (2024). https://doi.org/10.1186/s12967-024-05383-5
45. Evans, J. A. (Ed.). (2008). Frozen food science and technology. Blackwell Publishing. https://doi.org/10.1002/9781444302325
46. James, C., Purnell, G. & James, S.J. A Review of Novel and Innovative Food Freezing Technologies. Food Bioprocess Technol 8, 1616–1634 (2015). https://doi.org/10.1007/s11947-015-1542-8
47. Koritsoglou, K., Christou, V., Ntritsos, G., Tsoumanis, G., Tsipouras, M. G., Giannakeas, N., & Tzallas, A. T. (2020). Improving the Accuracy of Low-Cost Sensor Measurements for Freezer Automation. Sensors, 20(21), 6389. https://doi.org/10.3390/s20216389
48. Zheng, L., & Sun, D.-W. (2006). Innovative applications of power ultrasound during food freezing processes—a review. Trends in Food Science & Technology, 17(1), 16-23. https://doi.org/10.1016/j.tifs.2005.08.010
49. Richmond Scientific. (2022). Reducing the energy used by ULT freezers in the lab. Richmond Scientific. https://www.richmondscientific.com/reduce-energy-used-by-ult-freezers-in-the-lab
50. Robertson, J., Franzel, L., & Maire, D. (2017). Innovations in cold chain equipment for immunization supply chains. Vaccine, 35(17), 2252-2259. https://doi.org/10.1016/j.vaccine.2016.11.094

51. Legett, R. (2014). Field demonstration of high-efficiency ultra-low-temperature laboratory freezers. Energy Efficiency & Renewable Energy: US Department of Energy; 2014. Available from: https://www.energy.gov/sites/default/files/2014/11/f19/ult_ demo_ report.pdf
52. Faugeroux, D. (2016). Ultra-low temperature freezer performance and energy use tests. Office of Sustainability: University of California, Riverside. Retrieved from https://sustainability.ucsc.edu/engage/green-certified/green-labs/resources/Green%20Procurement/ucr2016_tempfreezertest.pdf
53. Keri, C. (2019). Chapter 13 - Recycling cooling and freezing appliances. In V. Goodship, A. Stevels, & J. Huisman (Eds.), Waste electrical and electronic equipment (WEEE) handbook (2nd ed., pp. 357-370). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-102158-3.00013-6
54. Powell, R. L. (2002). CFC phase-out: Have we met the challenge? Journal of Fluorine Chemistry, 114(2), 237-250. https://doi.org/10.1016/S0022-1139(02)00030-1
55. Tica, G., & Grubic, A. R. (2019). Mitigation of climate change from the aspect of controlling F-gases in the field of cooling technology. In IOP Conference Series: Materials Science and Engineering, 477, 012056. https://doi.org/10.1088/1757-899X/477/1/012056
56. Benhadid-Dib, S., & Benzaoui, A. (2012). Refrigerants and their environmental impact: Substitution of hydrochlorofluorocarbon (HCFC) and hydrofluorocarbon (HFC). Search for an adequate refrigerant. Energy Procedia, 18, 807-816. https://doi.org/10.1016/j.egypro.2012.05.096
57. Muir, E. B. (1990). Commercial refrigeration and CFCs. International Journal of Refrigeration, 13(2), 106-111. https://doi.org/10.1016/0140-7007(90)90009-L
58. Calderazzi, L., & Colonna di Paliano, P. (1997). Thermal stability of R-134a, R-141b, R-13I1, R-7146, R-125 associated with stainless steel as a containing material. International Journal of Refrigeration, 20(6), 381-389. https://doi.org/10.1016/S0140-7007(97)00043-1
59. Finberg, E. A., & Shiflett, M. B. (2021). Process designs for separating R-410A, R-404A, and R-407C using extractive distillation and ionic liquid entrainers. Industrial & Engineering Chemistry Research, 60(44). https://doi.org/10.1021/acs.iecr.1c02653
60. Zhang, W., Li, W., Cernicin, V., & Hrnjak, P. (2024). Theoretical and experimental investigation on the effects of internal heat exchangers on a reversible automobile R744 air-conditioning system under various operating conditions. Applied Thermal Engineering, 236(Part B), 121569. https://doi.org/10.1016/j.applthermaleng.2023.121569
61. Johnson, R. W. (2004). The effect of blowing agent choice on energy use and global warming impact of a refrigerator. International Journal of Refrigeration, 27(7), 794–799.
62. Lemmon, E. W. (2003). Pseudo-pure fluid equations of state for the refrigerant blends R-410A, R-404A, R-507A, and R-407C. International Journal of Thermophysics, 24, 991–1006. https://doi.org/10.1023/A:1025048800563
63. Liopis, R., Sánchez, D., Cabello, R., Catalán-Gil, J., & Nebot-Andrés, L. (2017). Experimental analysis of R-450A and R-513A as replacements of R-134a and R-507A in a medium temperature commercial refrigeration system. International Journal of Refrigeration, 84, 52-66. https://doi.org/10.1016/j.ijrefrig.2017.08.022
64. Liopis, R., Calleja-Anta, D., Sánchez, D., Nebot-Andrés, L., Catalán-Gil, J., & Cabello, R. (2019). R-454C, R-459B, R-457A and R-455A as low-GWP replacements of R-404A: Experimental evaluation and optimization. International Journal of Refrigeration, 106, 133-143. https://doi.org/10.1016/j.ijrefrig.2019.06.013
65. Komarov, S. G., & Stankus, S. V. (2011). Experimental study of speed of sound in gaseous refrigerant R-507A. High Temperature, 49, 150–153. https://doi.org/10.1134/S0018151X1101010X
66. Reddy, V. S., Panwar, N. L., & Kaushik, S. C. (2012). Exergetic analysis of a vapour compression refrigeration system with R134a, R143a, R152a, R404A, R407C, R410A, R502, and R507A. Clean Technologies and Environmental Policy, 14, 47–53. https://doi.org/10.1007/s10098-011-0374-0
67. Graham, M., Samuel, G., & Farley, M. (2024). Roadmap for low-carbon ultra-low temperature storage in biobanking. Journal of Translational Medicine, 22, 747. https://doi.org/10.1186/s12967-024-05383-5

68. Choi, T. Y., Kim, Y. J., Kim, M. S., & Ro, S. T. (2000). Evaporation heat transfer of R-32, R-134a, R-32/134a, and R-32/125/134a inside a horizontal smooth tube. International Journal of Heat and Mass Transfer, 43(19), 3651-3660. https://doi.org/10.1016/S0017-9310(00)00005-3
69. Ong, K. S., & Haider-E-Alahi, M. (2003). Performance of a R-134a-filled thermosyphon. Applied Thermal Engineering, 23(18), 2373-2381. https://doi.org/10.1016/S1359-4311(03)00207-2
70. Kim, C.-H., & Kim, N.-H. (2021). Condensation heat transfer and pressure drop of low GWP R-404A-alternative refrigerants (R-448A, R-449A, R-455A, R-454C) in a 7.0-mm outer-diameter horizontal microfin tube. International Journal of Refrigeration, 126, 181-194. https://doi.org/10.1016/j.ijrefrig.2021.01.025
71. Kim, M. S., Mulroy, W. J., & Didion, D. A. (1994). Performance evaluation of two azeotropic refrigerant mixtures of HFC-134a with R-290 (propane) and R-600a (isobutane). ASME Journal of Energy Resources Technology, 116(2), 148–154. https://doi.org/10.1115/1.2906020
72. Lee, B.-M., Gook, H.-H., Lee, S.-B., Lee, Y.-W., Park, D.-H., & Kim, N.-H. (2021). Condensation heat transfer and pressure drop of low GWP R-404A alternative refrigerants (R-448A, R-449A, R-455A, R-454C) in a 5.6 mm inner diameter horizontal smooth tube. International Journal of Refrigeration, 128, 71-82. https://doi.org/10.1016/j.ijrefrig.2020.12.025
73. Cho, K., & Tae, S.-J. (2000). Evaporation heat transfer for R-22 and R-407C refrigerant–oil mixture in a microfin tube with a U-bend. International Journal of Refrigeration, 23(3), 219-231. https://doi.org/10.1016/S0140-7007(99)00051-1
74. Cho, K., & Tae, S.-J. (2001). Condensation heat transfer for R-22 and R-407C refrigerant–oil mixtures in a microfin tube with a U-bend. International Journal of Heat and Mass Transfer, 44(11), 2043-2051. https://doi.org/10.1016/S0017-9310(00)00253-2
75. Lee, J. H., Bae, S. W., Bang, K. H., & Kim, M. H. (2002). Experimental and numerical research on condenser performance for R-22 and R-407C refrigerants. International Journal of Refrigeration, 25(3), 372-382. https://doi.org/10.1016/S0140-7007(01)00012-3
76. Guilherme, Í. F., Pico, D. F. M., Santos, D. D. O., & Bandarra Filho, E. P. (2022). A review on the performance and environmental assessment of R-410A alternative refrigerants. Journal of Building Engineering, 47, 103847. https://doi.org/10.1016/j.jobe.2021.103847
77. Ribeiro, R. P. P. L., Sosa, J. E., Araújo, J. M. M., Pereiro, A. B., & Mota, J. P. B. (2023). Vacuum swing adsorption for R-32 recovery from R-410A refrigerant blend. International Journal of Refrigeration, 150, 253-264. https://doi.org/10.1016/j.ijrefrig.2023.01.020
78. Calleja-Anta, D., Martínez-Ángeles, M., Nebot-Andres, L., Sánchez, D., & Llopis, R. (2024). Optimizing R152a/R600 and R290/R600 mixtures for superior energy performance in vapor compression systems: Promising alternatives to Isobutane (R600a). Applied Thermal Engineering, 247, 123070. https://doi.org/10.1016/j.applthermaleng.2024.123070
79. Liu, Z., Ji, S., Tan, H., Yang, D., & Cao, Z. (2023). An ultralow-temperature cascade refrigeration unit with natural refrigerant pair R290-R170: Performance evaluation under different ambient and freezing temperatures. Thermal Science and Engineering Progress. https://doi.org/10.1016/j.tsep.2023.102202
80. Udroiu, C.-M., Mota-Babiloni, A., & Navarro-Esbrí, J. (2022). Advanced two-stage cascade configurations for energy-efficient –80 °C refrigeration. Energy Conversion and Management, 267, 115907. https://doi.org/10.1016/j.enconman.2022.115907
81. Wen, M.-Y., & Ho, C.-Y. (2005). Evaporation heat transfer and pressure drop characteristics of R-290 (propane), R-600 (butane), and a mixture of R-290/R-600 in the three-lines serpentine small-tube bank. Applied Thermal Engineering, 25(17-18), 2921-2936. https://doi.org/10.1016/j.applthermaleng.2005.02.013
82. Chen, L.-S., Zawertailo, L., Piasecki, T. M., Kaprio, J., Foreman, M., Elliott, H. R., David, S. P., Bergen, A. W., Baurley, J. W., & Tyndale, R. F. (2018). Leveraging genomic data in smoking cessation trials in the era of precision medicine: Why and how. Nicotine & Tobacco Research, 20(4), 414–424. https://doi.org/10.1093/ntr/ntx097
83. Jones, A., Wolf, A., & Kwark, S. M. (2022). Refrigeration system development with limited charge of flammable refrigerant, R-290. Thermal Science and Engineering Progress, 34, 101392. https://doi.org/10.1016/j.tsep.2022.101392

84. Pilla, T. S., Sunkari, P. K. G., Padmanabhuni, S. L., Nair, S. S., & Dondapati, R. S. (2017). Experimental evaluation of mechanical performance of the compressor with mixed refrigerants R-290 and R-600a. Energy Procedia, 109, 113-121. https://doi.org/10.1016/j.egypro.2017.03.065
85. Jeon, S., Ko, J., Lee, H., & Jeong, J. H. (2025). Advanced model for a non-adiabatic capillary tube considering both subcooled liquid and non-equilibrium two-phase states of R-600a. International Journal of Refrigeration, 169, 140-151. https://doi.org/10.1016/j.ijrefrig.2024.10.031
86. Ye, G., Ye, M., Yang, J., Wu, X., Yan, Y., Guo, Z., & Han, X. (2024). Investigation on absorption and separation performance of R-32, R-125, R-134a, and R-1234yf refrigerants using EMIM-based ionic liquids. ACS Sustainable Chemistry & Engineering, 12(5). https://doi.org/10.1021/acssuschemeng.3c04730
87. Wang, N., Carlozo, M. N., Marin-Rimoldi, E., Befort, B. J., Dowling, A. W., & Maginn, E. J. (2023). Machine learning-enabled development of accurate force fields for refrigerants. Journal of Chemical Theory and Computation, 19(14). https://doi.org/10.1021/acs.jctc.3c00057
88. Midzic Kurtagic, S., Kadric, D., Alispahic, M., Blazevic, R., & Hadziahmetovic, H. (2022). Inventory of refrigerants in use for commercial purposes in BiH. In B. Katalinic (Ed.), Proceedings of the 33rd DAAAM International Symposium (pp. 143-150). DAAAM International. https://doi.org/10.2507/33rd.daaam.proceedings.020
89. Yang, Cheng-Min; Shen, Bo; Muneeshwaran, M; Nawaz, Kashif; Pickles, Ernest Calvin; and Hartnett, Christopher, "Performance Evaluation Of Various Configurations For Domestic Refrigerators With R-600a" (2024). International Refrigeration and Air Conditioning Conference. Paper 2656. https://docs.lib.purdue.edu/iracc/2656
90. Yang, M.-H., & Yeh, R.-H. (2022). Investigation of the potential of R717 blends as working fluids in the organic Rankine cycle (ORC) for ocean thermal energy conversion (OTEC). Energy, 245, 123317. https://doi.org/10.1016/j.energy.2022.123317
91. Fabris, F., Fabrizio, M., Marinetti, S., Rossetti, A., & Minetto, S. (2024). Evaluation of the carbon footprint of HFC and natural refrigerant transport refrigeration units from a life-cycle perspective. International Journal of Refrigeration, 159, 17-27. https://doi.org/10.1016/j.ijrefrig.2023.12.018
92. Kanbur, B. B., Kriezi, E. E., Markussen, W. B., Kærn, M. R., Busch, A., & Kristófersson, J. (2025). Framework for prediction of two-phase R-744 ejector performance based on integration of thermodynamic models with multiphase mixture CFD simulations. Applied Thermal Engineering, 258(Part C), 124888. https://doi.org/10.1016/j.applthermaleng.2024.124888
93. Lee, J.-H., Kim, J.-H., Heo, I.-Y., Yoon, J.-I., Son, C.-H., Nam, J.-W., Kim, H. J., Cha, S.-Y., & Seol, S.-H. (2024). Optimal charge amount for semiconductor chiller applying eco-friendly refrigerant R-744. Case Studies in Thermal Engineering, 59, 104461. https://doi.org/10.1016/j.csite.2024.104461
94. Macrì, C., De León, Á., & Flohr, F. (2024). Comparison of performance and efficiency of different refrigerants at high load conditions and their impact on CO2eq emissions. In CO2 Reduction for Transportation Systems Conference (pp. 1-7). Daikin Chemical Europe GmbH. https://doi.org/10.4271/2024-37-0029
95. Söylemez, E. (2024). Energy and Conventional Exergy Analysis of an Integrated Transcritical CO2 (R-744) Refrigeration System. Energies, 17(2), 479. https://doi.org/10.3390/en17020479
96. Babushok, V. I., & Linteris, G. T. (2024). Air humidity influence on combustion of R-1234yf (CF3CFCH2), R-1234ze(E) (trans-CF3CHCHF), and R-134a (CH2FCF3) refrigerants. Combustion and Flame, 262, 113352. https://doi.org/10.1016/j.combustflame.2024.113352
97. Leehey, M. H., Kujak, S., & Collins, C. (2024). Chemical stability of HFO and HCO refrigerants. In International Refrigeration and Air Conditioning Conference (Paper 2555). Purdue University. Retrieved January 4, 2025 from https://docs.lib.purdue.edu/iracc/2555
98. Miyawaki, K., & Shikazono, N. (2024). Experimental evaluation of NIR spectroscopic characteristics of liquid R32, R1234yf, and R454C refrigerants. International Communications in Heat and Mass Transfer, 156, 107633. https://doi.org/10.1016/j.icheatmasstransfer.2024.107633
99. Ha, S.-J., & Lee, J.-H. (2024). Heat transfer characteristics of R-1234yf, an eco-friendly alternative refrigerant, in condenser for semiconductor chiller. Applied Thermal Engineering, 239, 122106. https://doi.org/10.1016/j.applthermaleng.2023.122106

 doi:10.20944/preprints202505.2338.v1

100. Maqbool, S., & Maddali, R. (2024). Effect of system configuration on the performance of a hybrid air conditioning system based on R-1234yf. Applied Thermal Engineering, 236(Part B), 121624. https://doi.org/10.1016/j.applthermaleng.2023.121624
101. Kumar, A., Lakshmi, B. J., Yang, S.-Y., & Wang, C.-C. (2024). Effect of viscosity grade (POE) on the smooth-tube pool boiling performance with R-1234ze(E) refrigerant. Applied Thermal Engineering, 241, 122328. https://doi.org/10.1016/j.applthermaleng.2023.122328
102. Robaczewski, C., Leehey, M. H., & DeDeker, Z. (2024). Material compatibility of seal materials with low GWP refrigerants and lubricant. In International Compressor Engineering Conference (Paper 2826). Purdue University. https://docs.lib.purdue.edu/icec/2826
103. Six, P., Valtz, A., Zhou, Y., Yang, Z., & Coquelet, C. (2024). Experimental measurements and correlation of vapor–liquid equilibrium data for the difluoromethane (R32) + 1,3,3,3-tetrafluoropropene (R1234ze(E)) binary system from 254 to 348 K. Fluid Phase Equilibria, 581, 114072. https://doi.org/10.1016/j.fluid.2024.114072
104. Cavallini, A. (2020). The state-of-the-art on refrigerants. In Journal of Physics: Conference Series (Vol. 1599, p. 012001). IOP Publishing Ltd. https://doi.org/10.1088/1742-6596/1599/1/012001
105. Mickoleit, E., Breitkopf, C., & Jäger, A. (2021). Influence of equations of state and mixture models on the design of a refrigeration process. International Journal of Refrigeration, 121, 193-205. https://doi.org/10.1016/j.ijrefrig.2020.10.017
106. Narute, S., Joshi, K., Rane, V., & Kokate, P. (2021). A brief review on development of refrigerants and their applications. International Research Journal of Engineering and Technology (IRJET), 8(7), 4885. http://www.irjet.net
107. Long, N. V. D., Lee, D. Y., Han, T. H., & et al. (2020). Purification of R-12 for refrigerant reclamation using existing industrial-scale batch distillation: Design, optimization, simulation, and experimental studies. Korean Journal of Chemical Engineering, 37, 1823–1828. https://doi.org/10.1007/s11814-020-0631-1
108. Naskar, R., & Mandal, R. (2023). A competitive study of all natural refrigerants implementation on 1.5-ton domestic air conditioners using Cool Pack software. Industrial Engineering Journal, 52(9), No. 1. ISSN: 0970-2555.
109. Petrick, G.M. (2006). The arbiters of taste: producers, consumers, and the industrialization of taste in America, 1900–1960. Doctoral dissertation, University of Delaware.
110. McLinden, M. O., & Huber, M. L. (2020). (R)Evolution of refrigerants. Journal of Chemical & Engineering Data, 65(9), 4176–4193. https://doi.org/10.1021/acs.jced.0c00338
111. Nederhand, R. J., Droog, S., Kluft, C., Simoons, M. L., de Maat, M. P., & Investigators of the EUROPA trial (2003). Logistics and quality control for DNA sampling in large multicenter studies. Journal of thrombosis and hemostasis: JTH, 1(5), 987–991. https://doi.org/10.1046/j.1538-7836.2003.00216.x
112. Nasarabadi, S., Hogan, M., & Nelson, J. (2018). Biobanking in precision medicine. Current Pharmacology Reports, 4, 91–101. https://doi.org/10.1007/s40495-018-0123-8
113. She, R. C., & Petti, C. A. (2015). Procedures for the storage of microorganisms. In J. H. Jorgensen, K. C. Carroll, G. Funke, M. A. Pfaller, M. L. Landry, S. S. Richter, & D. W. Warnock (Eds.), Manual of clinical microbiology (11th ed.). American Society for Microbiology Press. https://doi.org/10.1128/9781555817381.ch11
114. Karim, A. S., & Jewett, M. C. (2018). Cell-free synthetic biology for pathway prototyping. In Methods in enzymology (Vol. 608, pp. 31-57). Academic Press. https://doi.org/10.1016/bs.mie.2018.04.029
115. Landor, L. A. I., Stevenson, T., Mayers, K. M. J., & et al. (2024). DNA, RNA, and prokaryote community sample stability at different ultra-low temperature storage conditions. Environmental Sustainability, 7, 77–83. https://doi.org/10.1007/s42398-023-00297-2
116. Bao, J., & Zhao, L. (2016). Experimental research on the influence of system parameters on the composition shift for zeotropic mixture (isobutane/pentane) in a system occurring phase change. Energy Conversion and Management, 113, 1-15. https://doi.org/10.1016/j.enconman.2016.01.005
117. Shaw, G. F. (2021). The Low Down on Ultralow Temperature Freezers. Endocrine News,40+. Retrieved February 9, 2025 from https://link.gale.com/apps/doc/A688004802/AONE?u=anon~103b3ad0&sid=googleScholar&xid=d263f033

118. Ajmani, P. S. (2020). Storage of blood. In Immunohematology and blood banking (pp. 1-15). Springer. https://doi.org/10.1007/978-981-15-8435-0_4
119. Sato, K., Tamaki, K., Okajima, H., & Katsumata, Y. (1988). Long-term storage of blood samples as whole blood at extremely low temperatures for methemoglobin determination. Forensic science international, 37(2), 99–104. https://doi.org/10.1016/0379-0738(88)90098-9
120. Tang, W., Hu, Z., Muallem, H., & Gulley, M. L. (2012). Quality assurance of RNA expression profiling in clinical laboratories. The Journal of molecular diagnostics: JMD, 14(1), 1–11. https://doi.org/10.1016/j.jmoldx.2011.09.003
121. Li, R., Johnson, R., Yu, G., McKenna, D. H., & Hubel, A. (2019). Preservation of cell-based immunotherapies for clinical trials. Cytotherapy, 21(9), 943-957. https://doi.org/10.1016/j.jcyt.2019.06.010
122. Shabihkhani, M., Lucey, G. M., Wei, B., Mareninov, S., Lou, J. J., Vinters, H. V., Singer, E. J., Cloughesy, T. F., & Yong, W. H. (2014). The procurement, storage, and quality assurance of frozen blood and tissue biospecimens in pathology, biorepository, and biobank settings. Clinical biochemistry, 47(4-5), 258–266. https://doi.org/10.1016/j.clinbiochem.2014.01.002
123. Kaushal, S., & Molinolo, A. (2021). Potential use of College of American Pathologists accredited biorepositories to bridge unmet need for medical refrigeration using ultralow temperature storage for COVID-19 vaccine or drug storage. Biopreservation and Biobanking, 19(3). https://doi.org/10.1089/bio.2020.016
124. Cabra, J., Castro, D., Colorado, J., Mendez, D., & Trujillo, L. (2017). An IoT approach for wireless sensor networks applied to e-health environmental monitoring. In Proceedings of the 2017 IEEE International Conference on Internet of Things (iThings), IEEE Green Computing and Communications (GreenCom), IEEE Cyber, Physical and Social Computing (CPSCom), and IEEE Smart Data (SmartData) (pp. 578-583). IEEE. https://doi.org/10.1109/iThings-GreenCom-CPSCom-SmartData.2017.91
125. Samuel, G., & Sims, J. M. (2023). Drivers and constraints to environmental sustainability in UK-based biobanking: Balancing resource efficiency and future value. BMC Medical Ethics, 24(1).
126. Isaacs, E., & Schmelz, M. (2017). Come in out of the cold: Alternatives to freezing for microbial biorepositories. Clinical Microbiology Newsletter, 39(4), 27-34. https://doi.org/10.1016/j.clinmicnews.2017.01.005
127. Hutchison, J. R., Brooks, S. M., Kennedy, Z. C., Pope, T. R., Deatherage Kaiser, B. L., Victry, K. D., Warner, C. L., Oxford, K. L., Omberg, K. M., & Warner, M. G. (2019). Polysaccharide-based liquid storage and transport media for non-refrigerated preservation of bacterial pathogens. PLOS ONE, 14(9), Article e0221831. https://doi.org/10.1371/journal.pone.0221831
128. Grasedieck, S., Schöler, N., Bommer, M., Niess, J. H., Tumani, H., Rouhi, A., Bloehdorn, J., Liebisch, P., Mertens, D., Döhner, H., Buske, C., Langer, C., & Kuchenbauer, F. (2012). Impact of serum storage conditions on microRNA stability. Leukemia, 26(11), 2414–2416. https://doi.org/10.1038/leu.2012.106
129. Groelz, D., Sobin, L., Branton, P., Compton, C., Wyrich, R., & Rainen, L. (2013). Non-formalin fixative versus formalin-fixed tissue: a comparison of histology and RNA quality. Experimental and molecular pathology, 94(1), 188–194. https://doi.org/10.1016/j.yexmp.2012.07.002
130. Lou, J. J., Mirsadraei, L., Sanchez, D. E., Wilson, R. W., Shabihkhani, M., Lucey, G. M., Wei, B., Singer, E. J., Mareninov, S., & Yong, W. H. (2014). A review of room temperature storage of biospecimen tissue and nucleic acids for anatomic pathology laboratories and biorepositories. Clinical Biochemistry, 47(4–5), 267-273. https://doi.org/10.1016/j.clinbiochem.2013.12.011
131. Shehu, D., Kim, M.-O., Rosendo, J., Krogan, N., Morgan, D. O., & Guglielmo, B. J. (2024). Institutional conversion to energy-efficient ultra-low freezers decreases carbon footprint and reduces energy costs. Biopreservation and Biobanking. https://doi.org/10.1089/bio.2024.007
132. Davis, A., Graves, S., & Murray, T. (2001). The Biophile sample process management system — Automated sample access at ultra low temperatures. SLAS TECHNOLOGY: Translating Life Sciences Innovation, 6(6). https://doi.org/10.1016/S1535-5535-04-00161
133. Kasi, P., & Cheralathan, M. (2021). Review of cascade refrigeration systems for vaccine storage. Journal of Physics: Conference Series, 2054, Article 012041. https://doi.org/10.1088/1742-6596/2054/1/012041

134. Bakaltcheva, I., & Reid, T. (2003). Effects of blood product storage protectants on blood coagulation. Transfusion Medicine Reviews, 17(4), 263-271. https://doi.org/10.1016/S0887-7963(03)00040-3
135. Greening, D. W., Glenister, K. M., Sparrow, R. L., & Simpson, R. J. (2010). International blood collection and storage: Clinical use of blood products. Journal of Proteomics, 73(3), 386-395. https://doi.org/10.1016/j.jprot.2009.07.011
136. Hess, J. R. (2010). Conventional blood banking and blood component storage regulation: Opportunities for improvement. Blood Transfusion, 8(Suppl 3), s9-15. https://doi.org/10.2450/2010.003S
137. Radin, J. (2017). Life on ice: A history of new uses for cold blood. Chicago: The University of Chicago Press.
138. Sperling, S., Vinholt, P. J., Sprogøe, U., Yazer, M. H., Frederiksen, H., & Nielsen, C. (2019). The effects of storage on platelet function in different blood products. Hematology, 24(1), 89-96. https://doi.org/10.1080/10245332.2018.1516599
139. Du, C., Xu, J., Song, H., Tao, L., Lewandowski, A., Ghose, S., Borys, M. C., & Li, Z. J. (2019). Mechanisms of color formation in drug substance and mitigation strategies for the manufacture and storage of therapeutic proteins produced using mammalian cell culture. Process Biochemistry, 86, 127-135. https://doi.org/10.1016/j.procbio.2019.08.013
140. Kao, G. S., Kim, H. T., Daley, H., Ritz, J., Burger, S. R., Kelley, L., Vierra-Green, C., Flesch, S., Spellman, S., Miller, J., & Confer, D. (2011). Validation of short-term handling and storage conditions for marrow and peripheral blood stem cell products. Transfusion, 51(5), 1129-1138. https://doi.org/10.1111/j.1537-2995.2010.02758.x
141. Sugrue, M. W., Hutcheson, C. E., Fisk, D. D., Roberts, C. G., Mageed, A., Wingard, J. R., & Moreb, J. S. (2009). The effect of overnight storage of leukapheresis stem cell products on cell viability, recovery, and cost. Journal of Hematotherapy, 7(5). https://doi.org/10.1089/scd.1.1998.7.431
142. Jewell, S. D., Srinivasan, M., McCart, L. M., Williams, N., Grizzle, W. H., LiVolsi, V., MacLennan, G., & Sedmak, D. D. (2002). Analysis of the molecular quality of human tissues: an experience from the Cooperative Human Tissue Network. American journal of clinical pathology, 118(5), 733–741. https://doi.org/10.1309/VPQL-RT21-X7YH-XDXK
143. Mager, S. R., Oomen, M. H. A., Morente, M. M., Ratcliffe, C., Knox, K., Kerr, D. J., Pezzella, F., & Riegman, P. H. J. (2007). Standard operating procedure for the collection of fresh frozen tissue samples. European Journal of Cancer, 43(5), 828-834. https://doi.org/10.1016/j.ejca.2007.01.002
144. Troyer, D. (2008). Biorepository Standards and Protocols for Collecting, Processing, and Storing Human Tissues. In: Liu, B.CS., Ehrlich, J.R. (eds) Tissue Proteomics. Methods in Molecular Biology™, vol 441. Humana Press. https://doi.org/10.1007/978-1-60327-047-2_13
145. International Society for Biological and Environmental Repositories (ISBER). (2008). Collection, storage, retrieval, and distribution of biological materials for research. Cell Preservation Technology, 6(1). https://doi.org/10.1089/cpt.2008.9997
146. Wolf, L. E., Bouley, T. A., & McCulloch, C. E. (2010). Genetic research with stored biological materials: Ethics and practice. IRB, 32(2), 7-18. Retrieved April 1, 2025 from https://pmc.ncbi.nlm.nih.gov/articles/PMC3052851/
147. Zimkus, B. M., & Ford, L. S. (2014). Best practices for genetic resources associated with natural history collections: Recommendations for practical implementation. Collection Forum, 28(1-2), 77–112. https://doi.org/10.14351/0831-0005-28.1.77
148. Elliott, M. A., & Halbert, G. W. (2005). Maintaining the cold chain shipping environment for Phase I clinical trial distribution. International Journal of Pharmaceutics, 299(1–2), 49-54. https://doi.org/10.1016/j.ijpharm.2005.04.032
149. Harada, L. M., Rodrigues, E. F., Ferreira, W. de P., Maniçoba da Silva, A., & Kawamoto Júnior, L. T. (2016). Storage management of clinical research supplies of a phase IIB/III, national, multi-centre, double-blind and randomized study. Brazilian Journal of Operations & Production Management, 13, 430-441.
150. Li, Q. C., Qiu, F., Cohen, K., & et al. (2012). Best practices for drug substance stress and stability studies during early-stage development Part I—Conducting drug substance solid stress to support phase Ia clinical trials. Journal of Pharmaceutical Innovation, 7, 214–224. https://doi.org/10.1007/s12247-012-9136-8

151. Lloyd, J., Lydon, P., Ouhichi, R., & Zaffran, M. (2015). Reducing the loss of vaccines from accidental freezing in the cold chain: the experience of continuous temperature monitoring in Tunisia. Vaccine, 33(7), 902–907. https://doi.org/10.1016/j.vaccine.2014.10.080
152. Liu, Z., Bai, M., Tan, H., Ling, Y., & Cao, Z. (2023). Experimental test on the performance of a −80 °C cascade refrigeration unit using refrigerants R290-R170 for COVID-19 vaccines storage. Journal of Building Engineering, 63(A), 105537. https://doi.org/10.1016/j.jobe.2022.105537
153. Groover, K., Kulina, L., & Franke, J. (2009). Effect of increasing thermal mass on chamber temperature stability within ultra-low freezers in bio-repository operations. https://biospecimens.cancer.gov/meeting/brnsymposium/2009/docs/posters/poster%2015%20groover.pdf
154. Rogers, J., Carolin, T., Vaught, J., & Compton, C. (2011). Biobankonomics: A taxonomy for evaluating the economic benefits of standardized centralized human biobanking for translational research. JNCI Monographs, 2011(42), 32–38.
155. Labmode. (2023). Nordic Modular ULT Freezer System. Labmode. https://labmode.co.uk/product/nordic-modular-ult-freezer-system/
156. International Organization for Standardisation. (2018). ISO 14067:2018 - Greenhouse Gases-Carbon Footprint of Products—Requirements and Guidelines for Quantification. International Organization for Standardisation.
157. Muenz, R. (2021). How to operate and maintain an ultralow temperature freezer. Lab Manager. https://www.labmanager.com/big-picture/lab-ultralow-cold-storage/how-to-operate-and-maintain-an-ultralow-temperature-freezer-24970
158. Pharmaceutical Inspection Co-operation Scheme. (2023). Guide to good manufacturing practice for medicinal products part I (PE 009-17). PIC/S Secretariat. Retrieved April 6, 2025, from https://www.picscheme.org
159. Tsimpoukis, D., Syngounas, E., Bellos, E., Koukou, M., Tzivanidis, C., Anagnostatos, S., & Vrachopoulos, M. G. (2024). Data-driven energy efficiency comparison between operating R744 and R448A supermarket refrigeration systems based on hybrid experimental-simulation analysis. Thermal Science and Engineering Progress, 53, 102776. https://doi.org/10.1016/j.tsep.2024.102776
160. McColloster, P. J., & Martin-de-Nicolas, A. (2014). Vaccine refrigeration: thinking outside of the box. Human vaccines & immunotherapeutics, 10(4), 1126–1128. https://doi.org/10.4161/hv.27660
161. Lucas, P., Pries, J., Wei, S., & Wuttig, M. (2022). The glass transition of water, insight from phase change materials. Journal of Non-Crystalline Solids, 1(14), 100084.
162. Li, Y., Pan, X., Liao, X., & Xing, Z. (2022). A data-driven energy management strategy based on performance prediction for cascade refrigeration systems. International Journal of Refrigeration, 136, 114-123. https://doi.org/10.1016/j.ijrefrig.2022.01.012
163. Gumapas, L. A. M., & Simons, G. (2013). Factors affecting the performance, energy consumption, and carbon footprint for ultra low temperature freezers: Case study at the National Institutes of Health. World Review of Science, Technology and Sustainable Development, 10(123), 129.
164. Koncept Media. (n.d.). Model K66 HPL [PDF]. Retrieved April 10, 2025, from https://www.konceptmedia.hr/admin/dokumenti/docfiles/model-K66-HPL.pdf
165. Stirling Ultracold. (n.d.). SU780XLE operating manual (Version 061824P). Retrieved April 10, 2025, from https://www.stirlingultracold.com/wp-content/uploads/2024/06/SU780XLE_Operating_Manual_Stirling_Ultracold_061824P.pdf
166. Klinge Corporation. (2019). PTI NMF-372 Prod 063 Rev C [PDF]. Retrieved April 10, 2025, from https://klingecorp.com/wp-content/uploads/2019/09/PTI-Form-NMF-372-Prod-063-Rev-C.pdf
167. WHO. (2014). Temperature mapping of storage areas. World Health Organization, Technical Report Series, No. 961, 2011 Annex 9.
168. WHO. (2015). Temperature mapping of storage areas. World Health Organization, Technical Report Series, No. 961, 2011, Supplement 8.
169. Human Tissue Authority. (2023). How licensing works under the Human Tissue Quality and Safety Regulations. Human Tissue Authority. https://www.hta.gov.uk/guidance-professionals/licences-roles-and-fees/licensing/how-licensing-works-under-human-tissue

 doi:10.20944/preprints202505.2338.v1

170. European Commission. (2011). Eudralex - volume 4: Good manufacturing practice (GMP) guidelines. Retrieved April 10, 2025, from URL https://health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-4_en.
171. U.S. Food and Drug Administration. (2024a). Title 21--Food and drugs, Chapter I--Food and Drug Administration, Department of Health and Human Services, Subchapter A - General, Part 11 Electronic records; electronic signatures. Retrieved April 10, 2025, from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=11.
172. European Commission. (2015). Guidelines of 19 March 2015 on principles of good distribution practice of active substances for medicinal products for human use (Text with EEA relevance) (2015/C 95/01). Retrieved April 10, 2025, from URL https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52015XC0321(01)
173. European Parliament & Council. (2000). Directive 2000/54/EC of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). OJ L 262, 17.10.2000, pp. 21–45. Retrieved April 10, 2025 from http://data.europa.eu/eli/dir/2000/54/oj
174. European Medicines Agency. (2016). Guideline on process validation for finished products - information and data to be provided in regulatory submissions (EMA/CHMP/CVMP/QWP/BWP/70278/2012-Rev1,Corr.1). Retrieved April 10, 2025 from https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-process-validation-finished-products-information-data-be-provided-regulatory-submissions_en.pdf.
175. European Parliament & Council. (2009). Directive 2009/41/EC of 6 May 2009 on the contained use of genetically modified micro-organisms (Recast) (Text with EEA relevance). OJ L 125, 21.5.2009, pp. 75–97. Retrieved April 09, 2025 from http://data.europa.eu/eli/dir/2009/41/oj
176. Evans, A. (2022). ULT Freezer Best Practice: Impacts. Green Light Labs. Retrieved April 09, 2025 from https://www.scientificlabs.co.uk/file/1991/Defrost%20the%20Freezer%20Regularly%20to%20Prevent%20a%20Build-up%20of%20Frost.
177. U.S. Food and Drug Administration. (2024b). Title 21--Food and drugs, Chapter I--Food and Drug Administration, Department of Health and Human Services, Subchapter C - Drugs: General, Part 210 Current good manufacturing practice in manufacturing, processing, packing, or holding of drugs; general. Retrieved from URL The American Society of Mechanical Engineers (2024). Bioprocessing Equipment. Retrieved April 15, 2025 from https://files.asme.org/Catalog/Codes/PrintBook/35606.pdf.
178. Kypraiou, C. (2025). Comparative Analysis of Temperature Uniformity and Efficiency in Low Temperature Refrigeration Systems of Hermetic Compressor, Free Piston Engine and Multicompressors (Unpublished master's thesis). Hellenic Open University, Master Thesis.