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REVIEW

Biobanking

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Abstract Biobanks, more formally known as biological resource centers (BRCs), form an "unsung" yet critical component of the infrastructures for scientific research, industry and conservation, without which much of the current scientific activity involving microbial cultures and cell-lines would be effectively impossible. BRCs are de facto depositories of "biological standards" holding taxonomic and other reference strains on which much of the associated published science and industrial standards are built and upon which some significant international commercial and ethical issues rely. The establishment and maintenance of BRCs is a knowledge- and skill-rich activity that in particular requires careful attention to the implementation of reliable preservation technologies and appropriate quality assurance to ensure that recovered cultures and other biological materials perform in the same way as the originally isolated culture or material. There are many types of BRC, which vary both in the kinds of material they hold and in their functional role. All BRCs are expected to provide materials and information of an appropriate quality for their intended use and work to standards relevant to those applications. There are important industrial, biomedical, and conservation issues that can only be addressed through effective and efficient operation of BRCs in the long term. This requires a high degree of expertise in the maintenance and management of collections of biological materials at ultra-low temperatures, or as freeze-dried material, to secure their long-term integrity and relevance for future research, development, and conservation.

Keywords Biobanks · Biological resource centers · Cell lines · Cryopreservation · Freeze-drying · Microorganisms · Quality assurance · Standardization

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Introduction

Collecting examples of different types of organisms has been the pursuit of scientists and amateur collectors for centuries. This activity was originally stimulated by man's curiosity regarding the natural diversity of 'his' environment, but for well over a century scientists have been collecting strains of animals, plants, and microorganisms with specific scientific and technical aims relating to taxonomy, infectious disease, biochemistry, and more recently for biotechnological and medical exploitation. The first service collection of microorganisms for industrial use was established by the Czech scientist, Dr. Frantisek Kral, in 1896 and collections of plants and other organisms developed based on the maintenance of examples of each strain or species under controlled laboratory or field conditions. Although it is possible to maintain some taxa such as *Chlorella vulgaris* CCAP 211/11B by serial transfer for many decades without any obvious genotypic, or phenotypic, changes [1], this is no longer standard practice for most organisms. In general, actively growing cultures may suffer from complications such as adaptation to the in vitro environment, genetic mutations, chromosomal abnormalities, mixing of strains by mis-transfer/mislabeling, contamination and accidental loss of cultures, or loss of

cultivars, in field collections due to disease or vermin attack. Clearly a mechanism for arresting growth to reduce such risks was needed.

The 1800s saw tremendous expansion in our scientific knowledge and engineering capabilities with the consequent development of new techniques including the compression of gases to the liquid state providing stable ultra-low temperature environments that enabled the field of cryobiology to develop rapidly. The ability to use ultra-low temperatures to prevent degradation of biological materials has probably been utilized by man for millennia and scientific discoveries in the 17th and 18th centuries paved the way for the successful preservation of prokaryotic and eukaryotic organisms in the 20th century, so they could be stored in a viable and unchanging state of "suspended animation" for indefinite periods. The preservation of bacteria and fungi had been established by pioneers from the 19th and early 20th centuries, with the subsequent work of researchers including Polge et al. [2] for preservation of animal cells and Sakai [3] for plant cells acting as key milestones in the development of cryopreservation processes. This pioneering work has been improved and refined with new approaches and fundamental research into cryobiology that has enabled the preservation of diverse and complex cell cultures and tissues (see [4] and references therein); however, it should be noted that many challenges remain and many microbial taxa, cell lines, and multicellular structures remain recalcitrant to current preservation methodologies.

Today culture collections, or more broadly, "biobanks" or biological resource centers (BRCs), have diverse remits, but commonly focus on discrete groups of organisms. These organizations are composed of many different kinds of activity: academic and "routine" testing laboratories, public service and private, government, and commercial activities that deliver important characterized cultures as "seed" stocks:

- as common sources of cultures reported in the literature,
- for the development of industrial processes,
- for the control of testing for quality assurance of products,
- as reference strains for biological assays and published scientific literature,
- as reference material in biomedical assays,
- as type and/or authentic strains for taxonomical studies, and
- as centers for conservation of biodiversity.

Industry invests large amounts of resource in developing specific clones or strains for manufacture and/or testing of products and needs access to biobanking expertise and services, which reside in the international BRC network. Some of these organizations have expertise in culture, preservation, and characterization of organisms that cannot be found elsewhere in academia or industry and information on capabilities and services can most readily be obtained by visiting their websites, contacting them directly, or via BRC organizations such as the World Federation of Culture Collections (WFCC) http://wdcm.nig.ac.jp/wfcc/. Industry also needs reliable and secure archiving mechanisms and equipment, and systems to assure traceability between stored materials, their provenance, and other critical characterization data. These activities are the primary focus and expertise of BRCs that should be utilized by those in industry and academia.

In this review, we have outlined some of the important principles and challenges involved in the establishment and long-term maintenance of collections of biological materials and cultures.

Fundamental Principles for Biological Resource Centers

There are four fundamental features of collections of biological materials that must be sustained to establish the value of stored material: (i) purity (freedom from contaminant organisms); (ii) authenticity (correct identity of each strain, ideally this also includes taxonomic affiliation in addition to confirmation of its culture collection accession/strain number); (iii) stability, including correct functional characteristics; and (iv) qualification data directly linked to each preserved stock of the culture.

The purity of a strain is critical to avoid erroneous data or performance of the culture (Fig. 1); however, in some situations establishment of pure cultures may not be possible as the organism may have a saprophytic relationship with other organisms and cannot survive as a "pure" culture (e.g., certain protists, saprophytic fungi), or can only be

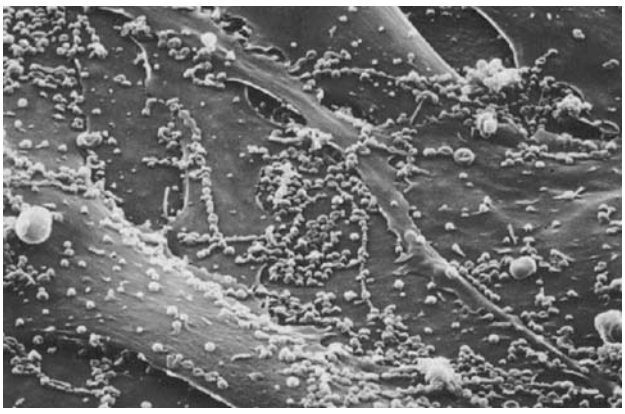

Fig. 1 Scanning electron micrograph of the surface of human fibroblasts showing numerous cells of mycoplasma organisms attached to the cell membranes (courtesy of Dr. D. Hockley, NIBSC)

preserved within their "host" cells, for example, the malaria parasite, or symbiotic fungi in orchid seeds [5, 6]. Authenticity is usually based on stable phenotypic or genotypic characteristics and invariably differs for each group of organisms. However, some DNA profiling (e.g., multi-locus DNA fingerprinting, Short Tandem Repeat analysis) [7] or gene sequencing/Bar coding techniques (e.g., cytochrome c oxidase sequencing [8], or "ribotyping" [9]) provide generic approaches that can be applied across a broad range of organisms in some cases. For cultures maintained in vitro, failure to maintain their phenotypic and genotypic features on serial passage is a serious concern as cultures (notably viruses, pathogenic bacteria, and cell lines [10]) are known to undergo irreversible changes if maintained in culture for long periods. Any preserved collection of cultures must have an associated and directly linked set of qualified data that demonstrates the value of the stored cultures in terms of their identity and characteristics and any links with other strains or published work. The security of this data is as important as the secure and stable storage of the strains themselves.

It is important that BRCs maintain practices to ensure that the samples of organisms that they hold and distribute sustain their important characteristics. This can be achieved through the adoption of working practices including establishment of master and distribution banks for each organism (Fig. 2) [11] and a robust quality control system to provide assurance that each bank prepared meets

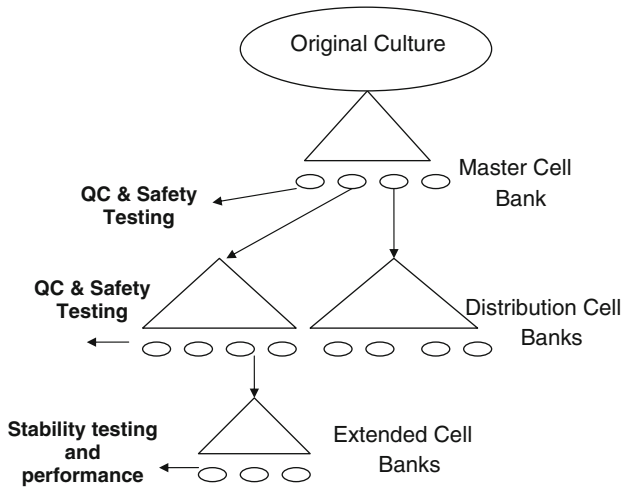

Fig. 2 Scheme of the "Master" and "Working" (or "Distribution") bank system. Each "bank" comprises a number of containers (⬭) each containing a sample of cryopreserved cells from a homogenously mixed single batch of harvested cells. Extended cell banks can also be established to enable testing of culture characteristics at different passages in parallel experiments. This banking scheme can be used to ensure that cultures are subjected to minimum passages in vitro before distribution to researchers. It can also act as the basis for a process map that can be used to establish a quality assurance system with appropriate traceability for bank preparation and quality control [12]

the criteria outlined above. The specific quality control regime will vary depending on the particular characteristics of the type of culture. A risk assessment will need to be performed relating to the history of each culture and the more general scientific experience with that culture type. Examples of such considerations are given in Table 1.

These requirements have been enshrined in best practice guidelines for culture collections such as the World Federation for Culture Collections' Standards [17]. These identify the minimum standards to ensure an appropriate level of quality of materials available from BRCs who generally seek to exceed them. In addition, there may be specialist guidance for the maintenance of the certain types of cell culture (e.g., http://www.stemcellforum.org/) and there are also legally binding regulations and ethical issues for some BRCs, as outlined in Table 2.

Long-term Roles for Biological Resource Centres

As already indicated the provision of cultures from BRCs is of little value unless these cultures are accompanied by information on their identity, provenance, and characteristics. Culture collections are important store houses of information on cultures which can be accessed by direct contact with individual collections or contacting microorganism database organizations such as MIRCEN at http://wdcm.nig.ac.jp/. Information resources can grow with time as work on cultures from BRCs is published and increasingly individual collections provide more comprehensive data, images, bibliographic data, and links to EMBL/Genbank directly from their websites, for example, the Culture Collection of Algae and Protozoa (CCAP) http://www.ccap.ac.uk. Such comprehensive "Knowledge–bases" are of increasing value to the scientific community and curated links between live material and bioinformatics data are of vital importance, particularly in the light of concerns over errors (taxonomic and others) in data published in the major depositories of molecular data [24, 25]. BRCs also engage in research and development on the technologies involved in the banking of cultures and are very often the only locations where this type of specialist R&D is maintained. Accordingly, BRCs are valuable sources of up-to-date information on topics such as biosafety, shipment, characterization, preservation, and taxonomy (see http://www.wfcc.info).

Many BRCs provide vital services for ex situ conservation of biodiversity through the preservation, storage, and documentation of endangered algae [26], plant tissues [27], seeds [28], and animal gametes and embryos [29, 30]. Such resource centers could potentially regenerate species that are lost to their natural habitat. The field of biotechnology and biomedicine is also reliant on long-term storage

Table 1 Standards that may apply to biological resource centres

Characteristics	Typical methods for bacterial strains	Typical methods for plant germplasm	Typical methods for animal cell lines
Purity	Broth and agar culture to reveal pure growth	Broth and agar culture to reveal absence of bacteria and fungi	Broth and agar culture to show no growth of bacteria and fungi [13] Tests for mycoplasma [13] and viral contamination (e.g., electron microscopy, molecular detection, inoculation of animals or test cell lines) [14]
Authenticity	Gram stain reaction, colony morphology, biochemical genotyping, biochemical reactions ribosomal gene sequence	Morphology of cells/structures, genotype, expression profile of 'secondary products', DNA genotype [15]	DNA fingerprint, karyology, isoenzyme analysis, bar coding [7]
Stability/functional capacity (cultures recovered from cell banks reproducibly show key functions)	Antibiotic susceptibility, absence of mutations by gene sequencing	Retain ability to regenerate plants, sustained expression of 'secondary products', absence of 'sports' [15, 16]	Antibody secretion by hybridomas, susceptibility to viruses, pluripotent differentiation in embryonic stem cell lines

Table 2 Standards for cell banking

Type of culture/application	Standards that may be applicable
Research and development	National regulations for handling, containment and disposal of pathogenic strains
	National regulations on genetically modified organisms
	Good cell culture practice [18]
Organisms deposited in support of patent applications	Budapest Treaty [19]
Plant cells and tissues	National and international regulations on import and export of pathogens (http://www.wfcc.info) and endangered species (http://www.cites.org/)
Animal cells and tissues	National and regulations on appropriate records of licenses, procedures, and staff training
	National and international regulations on import and export of pathogens and endangered species (see above)
Human cells and tissues for research	National regulations on ethical procurement and project approval
Human cells and tissues for transplantation and therapy	National and international regulations on ethical procurement, processing and storage [20]
Organisms and cell lines for manufacture of products for human therapy	International regulations for cell substrates [21, 22]
	International regulations on proliferation of biological warfare
Organisms and cell lines for testing purposes	International regulations and protocols for product testing (e.g. OECD Good Laboratory Practice [23]), Pharmacopeia monographs (http://www.usp.org/ or http://www.pheur.org/)

of viable cells to secure reliable seed stocks for the manufacture and testing of products. Furthermore, such long-term storage is critical to secure intellectual property by supporting patent applications where the preserved cells must remain viable for at least 30 years! [19, 31].

Assuring the Success of Long-term Storage

Although techniques such as culturing at sub-optimal temperatures [32], under low light for photoautotrophs [32], under mineral oil for fungi [33] are all commonly employed in BRCs, they all have limitations, not least in so far as they cannot guarantee phenotypic or genotypic stability. In general, long-term conservation of biological resources depends on rendering cell into a metabolically inactive state that ensures their long-term stability. This invariably depends on the removal of water, or making it unavailable for biological, and if possible chemical, activity. The most commonly employed approaches involve the use of freeze-drying or cryopreservation techniques.

Freeze-drying

Operationally one can define freeze-drying as a controllable method of dehydrating cells by vacuum desiccation [34]. Freeze-drying, or lyophilization, is widely used to conserve bacterial and fungal cultures in particular [25, 35]. Samples may be pretreated with a lyoprotectant, for example "skimmed" milk, or insositol (5%), cooled to convert the freezable solution water into ice, undergo sublimation of the ice under vacuum with "evaporation" of water from the sample and subsequent secondary drying removing moisture by desorption to a final moisture content of $\sim$1–2% [25, 35].

Although many workers regard freeze-drying as a relatively gentle and reliable method of preserving microbial cells, in reality it requires careful development and control and even then is still a potentially damaging process. The individual process stages induce a series of interrelated stresses, each of which can damage sensitive cells. Damage sustained during one step in the process may be exacerbated at succeeding stages in the process chain and even apparently trivial changes, such as a change in the type or dimensions of the storage vial, may be sufficient to derogate a successful process to one which is unacceptable [36]. Reducing temperature in the presence of ice formation is the first major stress imposed on the cells. Direct damage by ice is generally only injurious to larger or more complex cells [37]; however, when the cells are frozen at very fast rates the formation of intracellular ice may be induced within the cell [38]. Furthermore, a rise in solute concentration by freeze-concentration as ice forms, changes in pH and tonicity during the process of freeze-drying all have the potential to cause cell damage [39–41].

It is possible to maintain some lyophilized bacterial cultures for many decades (Table 3); however, in most cases cells are sensitive to thermal decay and will be influenced by storage temperature [42], moisture content [43–45], presence of reactive gases [46], light [36], free radicals [47], and background nuclear radiation [36]. Therefore, most major collections employing lyophilization have established procedures to undertake periodic viability checks and replacement of freeze-dried stock cultures.

Cryopreservation

Low-temperature/ultra-low temperature storage is increasingly the method of choice by BRCs to conserve materials as at low subzero temperatures (less than −135°C), little/no

Table 3 Examples of long-term viability of preserved biomaterials

Taxa/material	Stored	Storage duration	Comment	Reference
Various bacteria	FD	5–35 years	Survival	[59]
	LN	30	Survival	[60]
Various cyanobacteria	LN	>25 years	No obvious loss in viability	Rippka et al. (personal communication)
Yeast	FD	Up to 30 years	Survival	[61]
Schizo-saccharomyces pombe	LN	10 years	Unchanged genetically	[62]
Fungi	FD	>30 years	Good survival	[33]
	LN	>25 years	Good survival	Smith et al. (personal communication)
Various microalgae	LN	>20 years	No reduction in viability	[63]
Chlorella vulgaris	LN	>20 years	Unchanged genetically	[1]
Lettuce seeds	LN	>10 years	No loss in viability	[48]
			Half-life c. 3400 years	
Stem cells	LN	15 years	High efficiency of recovery	[64]
Human and sheep red blood cells	LN	12 years	No deterioration of function	[65]
Human hematopoietic cells	LN	14 years	Retain engraftment potential	[66]
Bovine sperm	LN	37 years	Normal motility and successful fertilization	[67]
Human sperm	LN	5 years	No statistical reduction in quality	[68]
	LN	21 years	Successful fertilization and live birth	[69]
Canine islets of langerham	LN	0.5 years	No reduction in insulin secretion	[70]
Sheep embryos	LN	13 years	No reduction in live births vs. 1 month storage	[71]

Note: FD, freeze-dried; LN, liquid nitrogen storage

further deterioration of stored material should occur and viability is effectively independent of storage duration for decades if not hundreds of years and a half-life for the viability of cryopreserved material has been estimated to be >3000 years [48]. Although many organisms can be successfully cryopreserved and stored at higher subzero temperatures, typically in a −80°C freezer [49, 50], for more complex eukaryotes in particular, viability levels rapidly fall during storage [28, 51].

Conventional Two-step Cryopreservation

As with freeze-drying, although some particularly sensitive materials are damaged by chilling to 0°C [52], most damage occurs when samples are cooled below 0°C, with the biological effects dominated by the freezing of water. Injury to the cells can occur at any stage of the process, but in general cell damage is either associated with the formation of intracellular ice, or the effects associated with increases in solute concentration [53, 54]. To avoid cryo-injury, cells are dehydrated as a result of a non-equilibrium vapor pressure gradient being formed at the point of extracellular ice nucleation, by controlled rate cooling. This results in the movement of unfrozen intracellular water out of the cell, thus reducing the amount of intracellular freezable water. The key factors influencing the degree of dehydration achieved include the rate of cooling, the temperature of ice nucleation, the terminal transfer temperature (this is most commonly −40°C, the temperature of homogeneous ice formation, although for some cell types including euglenoids −60°C has been employed [55]), before exposure to liquid nitrogen and the holding times at these respective steps. Successful traditional, controlled rate freezing requires the careful application/choice of colligative cryoprotectants, like glycerol or dimethyl sulfoxide (DMSO). These reduce the "solution" effects, resulting in a lower optimal cooling rate and generally increase the maximum survival levels obtained [52]. Cells are successfully cryopreserved when a critical balance between ice nucleation and dehydration is achieved, so that when plunged into liquid nitrogen any ice crystals that form are too small to cause damage, or they form an amorphous glass.

Vitrification

Vitrification is increasingly being applied as an alternative cryogenic approach to conserve higher plant germplasm as well as mammalian/human reproductive cells and tissues [30, 56]. Vitrification occurs when the viscosity of any solution reaching a sufficiently high value ($\sim 10^{13}$ poises) that causes it to behave like a solid, but without crystallization, that is, to become an amorphous "glass" [52]. Accordingly, cryopreservation using vitrification techniques results in solidification of water in the absence of crystallization, thus allowing cryogenic storage in the absence of ice. The high viscosities required may be achieved by evaporative desiccation (e.g., in sterile air streams, or over silica gel) with osmotic dehydration and/or the application of penetrating cryoprotectants. Plant vitrification involves the application of multiple-component chemical additives [57] such as Plant Vitrification Solution Number 2 (PVS2) [57], osmotic and evaporative dehydration and desiccation [58] with, or without, alginate encapsulation [57]. Vitrification is critically dependent upon the formation of a stable glassy state on both cooling and re-warming [52]. The latter can be a significant problem on storage if cryostat temperatures cannot be guaranteed, as in some cases spontaneous devitrification, with the formation of ice, can occur at temperatures greater than −135°C. A further limitation is the natural desiccation tolerance range of the germplasm to be cryopreserved and/or its tolerance to the chemicals employed in the vitrification solutions.

Experience and Critical Issues

It is often assumed that once an organism has been successfully preserved in a viable state in liquid nitrogen that it will remain viable indefinitely at ultra-low storage temperatures, and a review of the literature on cryopreservation and freeze-drying gives many examples of successful storage of cryopreserved organisms for periods of up to several decades (Table 3).

However, statements regarding sustained viability of cryopreserved cells are often based on the assumption that their storage temperature will be reliably maintained at the temperature of liquid nitrogen (−196°C). Clearly material stored in the vapor-phase will never achieve this temperature and the actual storage temperature may fluctuate due to variation in the levels of liquid nitrogen in storage vessels over time and the frequency of opening the vessel. Critically the storage temperature should not rise above the glass transition temperature (the temperature at which the water in an amorphous glass becomes unstable) of any vitrified material [36, 52]. Individuals searching for stored material may also move preserved preparations into ambient temperatures for brief periods, which, if repeated over time, could lead to loss of viability. In order to assure the long-term viability of stored cells and tissues, it is important to ensure that storage areas are well controlled with regular checks on liquid nitrogen levels and vessel filling rotas, and most importantly, appropriate staff training [21].

Storage Facilities

Having invested time and resources in the quality of banks of cell cultures, it is wise to provide a secure, clean, and stable environment for long-term storage which is also safe for laboratory staff to use [21]. Security for stored material is assured through adoption of appropriate management systems to restrict access to authorized personnel, appropriate alarms for nitrogen storage vessels and documented procedures for filling and maintenance of nitrogen storage. Monitoring in the form of temperature alarm systems with automatic notification of alarms to responsible personnel out of working hours and auditing to ensure correct maintenance and documentation are also important activities for BRC operation.

It is important to establish whether storage will be in the liquid or vapor-phase of nitrogen, or if electrical freezers (−100°C or below) are to be considered. In theory the liquid-phase of nitrogen provides the lowest and most stable storage temperature and is the method of choice for long-term storage. However, the risks of transmission of contaminants between storage vials should be considered as highlighted in past cases of patient deaths due to bone marrow contaminated by hepatitis virus during storage [72]. Vapor-phase storage may increase the risk of temperature cycling in stored materials, but is generally more convenient and safer for regular access to stored material than liquid-phase storage. Some manufacturers (e.g., CBS, USA) supply vapor-phase storage systems where liquid nitrogen is retained in the vessel walls, thus improving safety for laboratory staff, but providing little leeway if filling of the vessel with refrigerant is delayed. Electrical storage systems provide a very practical and maintenance-free low temperature storage solution. However, materials stored in such systems in a multi-user environment may suffer from regular disturbance to access material with the risk that critical materials may be lifted into ambient temperatures on a regular basis. Electrical freezers are also at risk where power supplies may not be reliable and even if power is reliable manufacturers often recommend liquid nitrogen, or carbon dioxide, back-up systems to cope with emergencies.

Appropriate facilities, equipment, and training in handling liquid nitrogen are vitally important for staff safety in cryostorage environments. In addition to the risks of cold burns and frostbite from contact with ultra-low temperature equipment, vapors and liquids, staff should also be made aware of the risks of asthma attack due to transient inhalation of nitrogen vapor and asphyxiation due to displacement of oxygen, as well as the possibility of ampoule explosion (due to trapped nitrogen liquid). The latter hazard is particularly important in enclosed storage areas since liquid nitrogen on warming to room temperature will rapidly expand to nearly 700× its original volume. The human senses will not alert laboratory workers when oxygen levels have become dangerously depleted and inhalation of atmospheres containing low oxygen can result in fainting and death within seconds. Accordingly, it is strongly recommended that staff working in liquid nitrogen handling areas are issued with personal oxygen monitors. Further information on cryogenic safety can be obtained from manufacturers (e.g., http://www.boccryospeed.co.uk/cryospeed/safety/gas_risks/cryogenics/). One further safety issue for consideration in longer term liquid nitrogen storage is the potential for oxygen to condense in liquid nitrogen and some facilities recommend routine replacement of all nitrogen in storage systems to avoid the build-up of oxygen and the risk of explosion.

Hazardous materials and those in quarantine should be stored separately to avoid the risk of transmission of infectious agents (see below) and it is also useful to separate material intended for archive storage and other material which will be accessed regularly to avoid temperature cycling and thus biological deterioration in material intended for long-term use.

Documentation of Stored Materials

Accurate records of stored materials are vital to enable retrieval of ampoules efficiently. They may also be a legal requirement where genetically modified, infectious or other hazardous materials are stored. Numerous commercial database systems are available specially designed for this purpose, but it is important to select a system which is flexible to the full range of user requirements. It is wise to have up-to-date hardcopy printouts or back-up electronic copies of these and it is helpful if amendments to storage records for additions or withdrawals can be made directly at the storage site to avoid transcription errors.

Researchers, biotechnologists, medical professions, and ecologists are subject to increasing regulation on the use of cultures derived from humans, animals, and indigenous or endangered species as outlined in Table 2. These issues can have significant legal implications for workers and accurate and traceable documentation of such cultures enabling linkage between the original donors/animals and sites of retrieval will be vital to deal with this new regulatory environment, and investment in BRC systems can provide the appropriate robust systems to assure preservation of the appropriate documentation as well as the cultures.

Other Issues for Long-term Storage

As with all equipment, storage refrigerators have a finite life span, therefore maintenance schedules and replacement timetables should be a part of any BRCs management

Table 4 Typical storage and transport conditions for different kinds of cryopreserved materials

Material	Storage conditions	Shipping conditions
Freeze-dried proteins, bacteria, and fungi	Generally stored at or below 4°C depending on stability	Can be stored at ambient temperature depending on thermal stability
Cryopreserved cells and organisms in vials or ampoules	Generally stored at temperatures below −100°C in electric freezers (−100 to −150°C) or in the liquid or vapor phase of liquid nitrogen (−160°C, −196°C). Storage is possible at −80°C but viability, particularly of eukaryotes, will decrease with time [48, 81]	Shipment can be made in dry ice packages (−80°C). It is vital to ensure sufficient dry ice is included to keep the material frozen until receipt. Good shipping companies will ensure that packages are topped up with dry ice
Cryopreserved tissues and cells for transplantation	Generally stored in the vapor phase (not submerged in liquid) of liquid nitrogen to avoid contamination	Special shipment containers are often used to maintain temperature of vapor phase LN
Cells preserved by vitrification	Stored below the glass transition temperature	Shipment must also be below the glass transition temperature (e.g. in dry shippers containing adsorbed nitrogen liquid). Higher temperatures, e.g. dry ice, will cause devitrification and loss of viability

strategy. As far as the authors are aware, no manufacturer currently suggests a life expectancy for their equipment, although warranties of up to 5 years are available on some storage vessels, and organizations including culture collections such as CCAP have vessels, which are >30 years old that are still in use for holding experimental material. Catastrophic failure of vessels is extremely rare, but is known to have occurred in BRCs in the UK, the US, and SE Asia. However, gradual deterioration of the vacuum has been observed by the authors. An additional management issue is that over time liquid nitrogen freezers become clogged with a build-up of "ice-sludge", which can accumulate microbial contamination from environmental sources [73]. Thus, long-term storage vessels will benefit from periodic cleaning to remove the ice-sludge (see also oxygen accumulation hazard above). However, moving stored material and/or inventory systems between vessels during the cleaning process is not without risk to the operative, or the thermal stability, and thus viability of the preserved materials.

It is also helpful to carefully disinfect recovered ampoules, to minimize inadvertent contamination of the samples [74]. The use of double sealing methods for ampoules or storage boxes will also help to provide protection against microbial contamination. Serious and lethal viral cross-infection of cells for medical transplantation from damaged containers in liquid nitrogen has been reported [72] and contamination can also result from the methods used to seal frozen stocks of infectious material [75, 76].

Natural radiation has also been considered a potential cause of loss of viability or mutation in stored cells and tissues. However, there does not appear to be any evidence for the adverse effects of long-term storage in well-maintained nitrogen vessels even for biological systems such as embryos that might be expected to be more sensitive to such effects [77].

A number of general recommendations for storage condition are given in Table 4. In addition, for important archive material it is wise to split such material between separate storage vessels and ideally to have an additional off-site storage location. It is clearly vital to monitor the quality of the storage environment for long-term sustained viability of stored cultures. Where much of the stored materials is of one type in terms of its potential stability in cryostorage, it may be helpful to establish "sentinel" banks of representative cells stored in locations prone to temperature cycling (e.g., the upper regions of storage inventory systems) that are recovered periodically to detect any trends in viability levels [78]. However, alternative physical temperature monitoring methods for key locations will generally be adequate and the technology has been developed to produce devices that could record and report the temperature experienced by individual vials or ampoules of cells in storage over time [79]. In some circumstances, accelerated thermal stability studies are used to predict the survival of stored biological materials at ultra-low temperature [28, 80, 81].

In some cases, particularly for cryopreserved fragile or otherwise problematic taxa, for example, eukaryotic microalgae, it is necessary to re-warm frozen samples and re-establish a new culture prior to distribution to an end-user. For many other materials, samples may be dispatched in a frozen or lyophilized state (see Table 4). Irrespective of whether samples are in a live, freeze-dried, or cryopreserved, format, all local, national, and international regulations should be observed. For fuller details see the websites listed in Table 5.

Table 5 Relevant websites on transportation and shipping regulations

Legislation/guidance	Website
A.D.R. rules (introduction)	http://www.unece.org/trans/danger/publi/adr/intro.htm
Air Transport Association	http://www.air-transport.org/
Canadian Transport	http://www.rural-gc.agr.ca/
DOT's Office of Hazardous Materials Safety	http://hazmat.dot.gov/
European Commission DGVII-Transport	http://europa.en.int/en/comm/dg07/index.html
International Air Transport Association (IATA)	http://www.IATA.org/cargo/dg/
	http://www.IATA.org/cargo/dg/links.htm
International Civil Aviation Organization (ICAO)	http://www.icao.int/index.html
International Maritime Organization (IMO)	http://www.imo.org/HOME.html
OECD	http://www.oecd.org/
Transport general	http://www.hazardousgoods.com/reguls/index.htm
	http://www.cefic.org
	http://www.tci-transport.fr
United Nations Committee of Experts on Transport of Dangerous Goods	http://www.tc.gc.ca/tdgoods/consult/unlink_e.HTM
United States International Trade Commission	http://www.usitc.gov
Universal Postal Union (UPU)	http://www.upu.int/
World Health Organisation	http://www.who.org/emc/biosafe/index.htm

Challenges for the Future

It is a common misconception that cryopreservation is a problem that has been solved. As we have described, numerous important strains and groups of organisms still cannot be cryopreserved successfully and, without reliably preserved master stocks, there is an ever present risk that such strains could be lost forever to science and industry. It is also apparent that standard preservation technologies may select out certain populations of cells/organisms. This has been observed in fungal cultures (e.g., respiration-deficient mutants of the yeast *Saccharomyces cerevisiae*, which has been demonstrated to lose plasmids during cryopreservation [82]) and in human cell preparations for research and biomedical applications (e.g., cord blood banking, bone marrow preservation) where certain cell types present in fresh cell preparations appear to be lost post-thaw. Thus, recovered cell cultures could display altered characteristics that may diminish their value in research and industrial applications and could lead to wasted time and investment in aberrant cultures. In biomedical applications, development of new methods that enable recovery of more of the native cell populations could also be important in improving transplant engraftment success and the development of new therapies. Vitrification methods requiring the use of "straws" and ultra-rapid freezing and warming for preservation of complex cellular structures (e.g., embryos, human embryonic stem cell lines, plant meristems, human tissues) are difficult to manage by novices, and vitrification methods enabling slower cooling and warming methods would enhance the reliability of these methods in users' hands. Not all cultures are readily scalable to deliver large cell banks for users. They may also suffer from lack of homogeneity, and approaches and techniques for reliable scale-up and assuring the homogeneity of large preserved stocks have yet to be delivered for certain cell culture (e.g., human embryonic stem cells, algae). The equipment for cryostorage is also in need of new technological developments. Traditional cryo-vials used to contain preserved cells do not deliver space efficient solutions for high-volume storage of very large numbers of aliquots, and storage vessels that could facilitate removal of ice-sludge in storage vessels and automated retrieval without warming of co-located samples are just two obvious examples. In addition, new radiofrequency identification devices (RFID) and ultra-low temperature microchip electronics are beginning to enable new technologies for non-invasive interrogation of inventories of stored materials and extensive data storage and temperature monitoring from within each individual container of frozen cells.

Conclusion

The availability of quality controlled and authentic biological materials and cultures through professional BRCs is a significant advantage for science, promoting standardization, efficiency, and laboratory safety. Culture strains become established in professional standards for industrial and biomedical work and may need to be stored stably for decades to support conservation programmes, production

processes, testing methods, and patents. BRCs may be challenged to provide cryostorage for large numbers of strains of organisms even when cryopreservation methods are not optimized for all organisms to be preserved. BRCs continue to provide sources of specialist advice and training in the skills of preservation and culture that are increasingly needed with the development and expansion of cell-based in vitro experimentation. However, continued governmental and industry support is vital.

The world is experiencing dramatic environmental change for which the end result is not clear and biological reference points will be vital. This is associated with a period of massive species extinctions and reduction in biodiversity. Furthermore, industry is increasingly recognizing the economic importance of appropriate biobanking of a broad range of biological cultures and materials. These and other activities need state-of-the-art science and technology in biobanking to deliver necessary systems and resources to manage the needs for preserved viable cultures in the long term. National BRCs with their unique blend of scientific knowledge, technical expertise, and collections of valuable microorganisms should be a resource of national pride for the science and industry of all countries. Coordinating their unique expertise and knowledge with the newly recognized needs for biobanking in industry, academia, and regulation will be a key element in meeting some of the critical future challenges for the exploration and exploitation of biodiversity.

References

1. Müller, J., Friedl, T., Hepperle, D., Lorenz, M., & Day, J. G. (2005). Distinction of isolates among multiple strains of *Chlorella vulgaris* (Chlorophyta, Trebouxiophyceae) and testing conspecificity with amplified fragment length polymorphism and ITS RDNA sequences. *Journal of Phycology, 41*, 1236–1247. doi:10.1111/j.1529-8817.2005.00134.x.
2. Polge, C., Smith, A. U., & Parkes, S. (1949). Revival of spermatozoa after dehydration at low temperatures. *Nature, 164*, 166. doi:10.1038/164666a0.
3. Sakai, A. (1966). Survival of plant tissues at super-low temperatures IV, cell survival with rapid cooling and rewarming. *Plant Physiology, 41*, 1050–1054.
4. Day, J. G., & Stacey, G. N. (2007). *Cryopreservation and freeze-drying protocols*. Totowa, NJ: Humana Press.
5. Mutetwa, S. M., & James, E. R. (1984). Cryopreservation of *Plasmodium chabaudi*. II. Cooling and warming rates. *Cryobiology, 21*, 552–558. doi:10.1016/0011-2240(84)90054-3.
6. Wood, C. B., Pritchard, H. W., & Miller, A. P. (2000). Simultaneous preservation of orchid seed and its fungal symbiont using encapsulation-dehydration is dependent on moisture content and storage temperature. *CryoLetters, 21*, 125–136.
7. Stacey, G. N., Byrne, E., & Hawkins, J. R. (2007). DNA fingerprinting and characterization of animal cell lines. In R. Poertner (Ed.), *Animal cell biotechnology: Methods and protocols* (2nd edn., pp. 123–145). Totowa, NJ: Humana Press.
8. Hebert, P. D., Cywinska, A., Ball, S. L., & de Waard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings. Biological Sciences, 270*, 313–321. doi:10.1098/rspb.2002.2218.
9. Bingen, E. H., Denamur, E., & Elion, J. (1994). Use of ribotyping in epidemiological surveillance of nosocomial outbreaks. *Clinical Microbiology Reviews, 7*, 311–327.
10. Stacey, G. N. (2002). Standardisation of cell lines. *Developments in Biologicals, 111*, 259–272.
11. Hay, R. J. (1988). The seed stock concept and quality control for cell lines. *Analytical Biochemistry, 171*, 225–237. doi:10.1016/0003-2697(88)90480-0.
12. Stacey, G. N. (2004). Validation of cell culture media components. *Human Fertility, 7*, 113–118.
13. Stacey, A., & Stacey, G. N. (2000). Routine quality control testing for cell cultures. In D. Kinchington & R. F. Schinazi (Eds.), *Methods in molecular medicine, vol. 24: Antiviral methods and protocols* (pp. 27–40). Totowa, NJ: Humana Press.
14. McLean, C. (2000). Contamination detection in animal cell culture. In R. Spier (Ed.), *Encyclopedia of cell technology* (pp. 586–609). New York: Wiley Interscience.
15. Harding, K. (2004). Genetic integrity of cryopreserved plant cells: A review. *CryoLetters, 25*, 3–22.
16. Stacey, G. N., Benson, E. E., & Lynch, P. T. (1999). Plant Genebanking: Agriculture, biotechnology and conservation. *Agro Food Industry Hi-Tech, 10*, 9–14.
17. WFCC. (1999). *World Federation for Culture Collections guidelines for the establishment and operation of collections of cultures of microorganisms*, 2nd edn. (ISBN 92-9109-043-3 available from Dr. A. Doyle, Secretary WFCC, The Wellcome Trust, 183 Euston Road, London NW1 2BE, UK).
18. Coecke, S., Balls, M., Bowe, G., Davis, J., Gstraunthaler, G., Hartung, T., et al. (2005). Guidance on good cell culture practice. A report of the second ECVAM task Force on Good Cell Culture Practice. *Alternatives to Laboratory Animals, 33*, 1–27.
19. Budapest Treaty Regulations. (1977). Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure. 277 (E), World Intellectual Property Organization, Geneva.
20. European Directive 2004/23/CE of the European parliament and the council of March 31st, relating to the establishment of quality and safety norms to donate, to obtain, to assess, to process, to preserve, to store and to distribute cells and human tissues.
21. World Health Organization Expert Committee on Biological Standardization and Executive Board (ECBS). (2005). Requirements for the use of animal cells as in vitro substrates for the production of biologicals. Technical Report Series 927, World Health Organization, Geneva.
22. ICH. (1997). Human Medicines Evaluation Unit: ICH Topic Q 5 D – Quality of biotechnological products: Derivation and characterization of cell substrates used for production of biotechnological/biological products. European Agency for the Evaluation of Medicinal Products, ICH Technical Co-ordination, London. http://www.eudra.org/emea.html.
23. OECD. (2004). Draft advisory document of the OECD working group on the application of GLP principles to in vitro studies. OECD, Paris.
24. Bridge, P. D., Roberts, P. J., Spooner, B. M., & Panchal, G. (2003). On the reliability of published DNA sequences. *The New Phytologist, 160*, 43–48. doi:10.1046/j.1469-8137.2003.00861.x.
25. Tindall, B. J. (2007). Vacuum drying and cryopreservation of prokaryotes. In J. G. Day & G. N. Stacey (Eds.), *Methods in molecular biology, vol. 368: Cryopreservation and freeze-drying protocols* (pp. 73–98). Totowa, NJ: Humana Press.
26. Watanabe, M. M., Nozaki, H., Kasaki, S., Sano, N., Kato, N., Omori, Y., et al. (2005). Threatened state of the Charales in the

lakes of Japan. In F. Kasai, K. Kaya, & M. M. Watanabe (Eds.), *Algal culture collections and the environment* (pp. 217–236). Kanagawa, Japan: Tokai Univ. Press.
27. Benson, E. E., Harding, K., & Johnston, J. W. (2007). Cryopreservation of shoot tips and meristems. In J. G. Day & G. N. Stacey (Eds.), *Methods in molecular biology, vol. 368: Cryopreservation and freeze-drying protocols* (pp. 163–184). Totowa, NJ: Humana Press.
28. Pritchard, H. W. (2007). Cryopreservation of desiccation-tolerant seeds. In J. G. Day & G. N. Stacey (Eds.), *Methods in molecular biology, vol. 368: Cryopreservation and freeze-drying protocols* (pp. 185–202). Totowa, NJ: Humana Press.
29. Curry, M. R. (2007). Cryopreservation of mammalian semen. In J. G. Day & G. N. Stacey (Eds.), *Methods in molecular biology, vol. 368: Cryopreservation and freeze-drying protocols* (pp. 303–312). Totowa, NJ: Humana Press.
30. Fuller, B. J., & Paynter, S. J. (2007). Cryopreservation of mammalian embryos. In J. G. Day & G. N. Stacey (Eds.), *Methods in molecular biology, vol. 368: Cryopreservation and freeze-drying protocols* (pp. 325–339). Totowa, NJ: Humana Press.
31. Stacey, G. N. (2004). Cell line banks in biotechnology and regulatory affairs. In B. Fuller, E. E. Benson, & N. Lane (Eds.), *Life in the frozen state* (pp. 437–452). Boca Ranton: CRC Press LLC.
32. Lorenz, M., Friedl, T., & Day, J. G. (2004). Perpetual maintenance of actively metabolizing microalgal cultures. In R. A. Andersen (Ed.), *Algal culturing techniques* (pp. 145–156). New York: Academic Press.
33. Smith, D., & Onions, A. H. S. (1994). *The preservation and maintenance of living fungi* (2nd edn.). Wallingford, UK: CAB International.
34. Adams, G. (2007). The principles of freeze-drying. In J. G. Day & G. N. Stacey (Eds.), *Methods in molecular biology, vol. 368: Cryopreservation and freeze-drying protocols* (pp. 1–14). Totowa, NJ: Humana Press.
35. Ryan, M. J., & Smith, D. (2007). Cryopreservation and freeze-drying of fungi employing centrifugal and shelf freeze-drying. In J. G. Day & G. N. Stacey (Eds.), *Methods in molecular biology, vol. 368: Cryopreservation and freeze-drying protocols* (pp. 127–140). Totowa, NJ: Humana Press.
36. Adams, G. D. J. (1995). *Freeze-drying—The integrated approach* (pp. 177–180). Pharmaceutical Manufacturing International.
37. Fleck, R. A., Day, J. G., Clarke, K. J., & Benson, E. E. (1999). Elucidation of the metabolic and structural basis for the cryopreservation recalcitrance of *Vaucheria sessilis*. *CryoLetters, 20*, 271–282.
38. Farrant, J. (1980). General observations on cell preservation. In M. Ashwood-Smith & J. Farrant (Eds.), *Low temperature preservation in medicine and biology* (pp. 1–18). Tonbridge Wells, UK: Pitman Medical.
39. Ashwood-Smith, M., & Farrant, J. (1980). *Low temperature preservation in medicine and biology*. Tonbridge Wells, UK: Pitman Medical.
40. Arakawa, T., Carpenter, F., Kita, Y. A., & Crowe, I. H. (1990). The basis for toxicity of certain cryoprotectants: a hypothesis. *Cryobiology, 27*, 401–415. doi:10.1016/0011-2240(90)90017-X.
41. Taylor, M. J. (1981). The meaning of pH at low temperature. *Cryobiology, 2*, 231–239.
42. Greiff, D., & Rightsel, W. A. (1965). Stabilities of suspensions of virus after vacuum sublimation and storage. *Cryobiology, 3*, 435–443.
43. Greiff, D. (1971). Protein structure and freeze-drying: The effects of residual moisture and gases. *Cryobiology, 8*, 145–152. doi:10.1016/0011-2240(71)90022-8.
44. Bellissent-Funel, M., & Teixera, Q. (1999). Structural and dynamic properties of bulk and confined water additives. In L. Rey & J. C. May (Eds.), *Freeze-drying/Lyophilization of pharmaceutical and biological products* (pp. 53–77). New York: Marcel Dekker.
45. Adams, G. D. J. (1990). Residual moisture and the freeze-dried product. In *Lyophilization technology handbook* (pp. 581–604). The Center for Professional Advancement, Academic Center, PO Box H, East Brunswick, NJ.
46. Cox, C. S. (1991). *Roles of maillard reactions in disease*. London: HMSO Publications.
47. Cowdery, S., Frey, M., Orlowski, S., & Gray, A. (1977). Stability characteristics of freeze-dried human live virus vaccines. In *International symposium on freeze-drying of biological products, vol. 36: Developments in biological standards* (pp. 297–303). Karger, Basel.
48. Walters, C., Wheeler, L., & Stanwood, P. C. (2004). Longevity of cryogenically stored seeds. *Cryobiology, 48*, 229–244. doi:10.1016/j.cryobiol.2004.01.007.
49. Dando, T. R., & Bousfield, I. J. (1991). Maintenance of industrial and marine bacteria. In B. E. Kirsop & A. Doyle (Eds.), *Maintenance of microorganisms and cell cultures* (2nd edn., pp. 57–64). London: Academic Press.
50. Jones, D., Pell, P. A., & Sneath, P. H. A. (1991). Maintenance of bacteria on glass beads at −60°C to −76°C. In B. E. Kirsop & A. Doyle (Eds.), *Maintenance of microorganisms and cell cultures* (2nd edn., pp. 45–50). London: Academic Press.
51. Brown, S., & Day, J. G. (1993). An improved method for the long-term preservation of *Naegleria gruberi*. *CryoLetters, 14*, 347–352.
52. Pegg, D. E. (2007). Principles of cryopreservation. In J. G. Day & G. N. Stacey (Eds.), *Methods in molecular biology, vol. 368: Cryopreservation and freeze-drying protocols* (pp. 39–58). Totowa, NJ: Humana Press.
53. Mazur, P. (2004). Principles of cryobiology. In B. Fuller, N. Lane, & E. E. Benson (Eds.), *Life in the frozen state* (pp. 3–66). Florida: CRC Press.
54. Meryman, H. I., Williams, R. J., St, J., & Douglas, M. (1977). Freezing injury from solution effects and its prevention by natural or artificial cryoprotection. *Cryobiology, 14*, 287–302. doi:10.1016/0011-2240(77)90177-8.
55. Morris, G. J., & Canning, C. E. (1978). The cryopreservation of *Euglena gracilis*. *Journal of General Microbiology, 108*, 27–31.
56. Benson, E. E. (2004). Cryoconserving algal and plant diversity: Historical perspectives and future challenges. In B. Fuller, N. Lane, & E. E. Benson (Eds.), *Life in the frozen state* (pp. 299–328). Florida: CRC Press.
57. Sakai, A. (2004). Plant cryopreservation. In B. Fuller, N. Lane, & E. E. Benson (Eds.), *Life in the frozen state* (pp. 329–346). Florida: CRC Press LLC.
58. Fabre, J., & Dereuddre, J. (1990). Encapsulation-dehydration: A new approach to cryopreservation of potato shoot-tips. *CryoLetters, 11*, 413–426.
59. Rudge, R. H. (1991). Maintenance of bacteria by freeze-drying. In B. E. Kirsop & A. Doyle (Eds.), *Maintenance of microorganisms and cell cultures* (2nd edn., pp. 31–44). London: Academic Press.
60. Hubalek, Z., & Kockova-Kratochvilova, A. (1982). Long term preservation of yeast cultures in liquid nitrogen. *Folia Microbiologica, 27*, 242–244. doi:10.1007/BF02877123.
61. Bond, C. (2007). Freeze-drying yeast cultures. In J. G. Day & G. N. Stacey (Eds.), *Methods in molecular biology, vol. 368: Cryopreservation and freeze-drying protocols* (pp. 99–108). Totowa, NJ: Humana Press.
62. Bond, C. (2007). Cryopreservation of yeast cultures. In J. G. Day & G. N. Stacey (Eds.), *Methods in molecular biology, vol. 368: Cryopreservation and freeze-drying protocols* (pp. 109–118). Totowa, NJ: Humana Press.

63. Day, J. G., Watanabe, M. M., Morris, G. J., Fleck, R. A., & McLellan, M. R. (1997). Long-term viability of preserved eukaryotic algae. *Journal of Applied Phycology, 9*, 121–127. doi:10.1023/A:1007991507314.
64. Broxmeyer, H. E., Srour, E. F., Hangoc, G., Cooper, S., Anderson, S. A., & Bodine, D. M. (2003). High-efficiency recovery of functional hematopoietic progenitor and stem cells from human cord blood cryopreserved for 15 years. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 5645–5650. doi:10.1073/pnas.0237086100.
65. Sputtek, A. (2007). Cryopreservation of red blood cells and platelets. In J. G. Day & G. N. Stacey (Eds.), *Methods in molecular biology, vol. 368: Cryopreservation and freeze-drying protocols* (pp. 283–302). Totowa, NJ: Humana Press.
66. Spurr, E. E., Wiggins, N. E., Marsden, K. A., Lowenthal, R. M., & Ragg, S. J. (2002). Cryopreserved human haematopoietic stem cells retain engraftment potential after extended (5–14 years) cryostorage. *Cryobiology, 44*, 210–217. doi:10.1016/S0011-2240(02)00027-5.
67. Leibo, S. P., Semple, M. E., & Kroetsch, T. G. (1994). In-vitro fertilization of oocytes by 37-year-old cryopreserved bovine spermatozoa. *Theriogenology, 42*, 1257–1262. doi:10.1016/0093-691X(94)90245-E.
68. Rofeim, O., & Gilbert, B. R. (2005). Long-term cryopreservation of human spermatozoa. *Fertility and Sterility, 84*, 536–537. doi:10.1016/j.fertnstert.2005.02.035.
69. Horne, G., Atkinson, A. D., Pease, E. H. E., Logue, J. P., Brison, D. R., & Lieberman, B. A. (2004). Live birth with semen cryopreserved for 21 years prior to cancer treatment. *Human Reproduction (Oxford, England), 19,* 1448–1449. doi:10.1093/humrep/deh249.
70. Chern, H. T., & Scharp, D. W. (1995). Successful long-term cryopreservation of highly purified canine islets. *European Surgical Research, 27*, 167–175. doi:10.1159/000129396.
71. Fogarty, N. M., Maxwell, W. M. C., Eppleston, J., & Evans, G. (2000). The viability of transferred sheep embryos after long-term cryopreservation. *Reproduction, Fertility, and Development, 12*, 31–37. doi:10.1071/RD00020.
72. Tedder, R. S., Zuckerman, M. A., Goldstone, A. H., et al. (1995). Hepatitis B transmission from a contaminated cryopreservation tank. *Lancet, 346*, 137–140. doi:10.1016/S0140-6736(95)91207-X.
73. Fountain, D., Ralston, M., Higgins, N., Gorlin, J. B., Uhl, L., Wheeler, C., et al. (1997). Liquid nitrogen freezers: A potential source of microbial contamination of hematopoietic stem cell components. *Transfusion, 37*, 585–591. doi:10.1046/j.1537-2995.1997.37697335152.x.
74. Day, J. G., & Brand, J. J. (2005). Cryopreservation methods for maintaining cultures. In R. A. Andersen (Ed.), *Algal culturing techniques* (pp. 165–187). New York: Academic Press.
75. Letur-Könirsch, H., Collin, G., Devaux, A., Madelenat, P., Brun-Vezinet, F., Feldmann, G., et al. (2003). Safety of cryopreservation straws for human gametes or embryos: A study with human immunodeficiency virus-1 under cryopreservation conditions. *Human Reproduction (Oxford, England), 18*, 140–144. doi:10.1093/humrep/deg001.
76. Maertens, A., Bourlet, T., Plotton, N., Pozzetto, B., & Levy, R. (2004). Validation of safety procedures for the cryopreservation of semen contaminated with hepatitis C virus in assisted reproductive technology. *Human Reproduction (Oxford, England), 19*, 1554–1557. doi:10.1093/humrep/deh275.
77. Glenister, P. H., Whittingham, D. G., & Lyon, M. F. (1984). Further studies on the effect of radiation during storage of frozen 8-cell mouse embryos at −196 degrees C. *Journal of Reproduction and Fertility, 70*, 229–234.
78. Stacey, G. N. (1999). Control of contamination in cell and tissue banks. *CryoLetters, 20*, 141–146.
79. Streit, S., Bock, F., Pirk, C. W., & Tautz, J. (2003). Automatic life-long monitoring of individual insect behaviour now possible. *Zoology (Jena), 106*, 169–171.
80. Kirkwood, T. B. L. (1984). Design and analysis of accelerated degradation tests for the stability of biological standards, III. Principles of design. *Journal of Biological Standardization, 12*, 215–224. doi:10.1016/S0092-1157(84)80056-6.
81. Ratajczak, M. Z., Kegnow, D. A., Kuczynski, W. I., Ratajczak, J., & Gewitz, A. M. (1994). The storage of cells from different tumor lines in a mechanical freezer at −80 degrees C. Comparison to cryopreservation in liquid nitrogen. *Materia Medica Polona. Polish Journal of Medicine and Pharmacy, 26*, 69–72.
82. Pearson, B. M., Jackman, P. J. H., Painting, K. A., & Morris, G. J. (1990). Stability of genetically manipulated yeasts under different cryopreservation regimes. *CryoLetters, 11*, 205–210.