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Biobanking in the 21st Century

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Preface

Over the last few decades, major discoveries have occurred in science and health disciplines which have had great impact on almost all the fields of biological sciences. Biobanking, inevitably, is a discipline that has also been affected and thus consequently necessitates a strategic re-evaluation pertaining to current status and running of biobanks and the incorporation of these new advances in human genome projects, bio-information technology, personalized medicine, stem cell, regenerative medicine, and so forth. These developments have created an urge for new strategies in many aspects of research and the ethics in biobanking of the future. There are increasing interests by both academia and industry to invest and collaborate in one of the ten ideas that are changing the world right now.

National population-based biobanks have been established in many countries and there are clear indications of openness, collaboration, and global networking among them. There are currently several excellent existing texts in the literature regarding biobanking. It was, therefore, a very challenging task to see how this volume could be recognized as a unique and informative text in the field. The thematic direction of this book is future biobanking in a global context and beyond the boundaries of countries. The book is not intended to have a specific target audience, but rather, to cover a broad range of topics and gain a far greater understanding as to the future of biobanking.

This book includes the views of some professional and lay organizations that have in the past been less frequently approached to express their role and contribution to biobanking in the future, such as nurses (Chap. 12), lay societies (Chap. 14), and to some extent pathologists (Chap. 5). I especially praise the Independent Cancer Patients' Voice based in the United Kingdom who shared the voice of patients with us. Also, directors of two disease-specific biobanks, i.e., breast cancer (Chap. 6) and neurological diseases (Chap. 7), discuss their objectives and challenges for the future.

Four chapters of the book, 8–11, detail the development of biobanks and biorepositories in four culturally, and economically different parts of the world: Canada, Brazil, China, and Jordan. I am excited and thankful to have these chapters side by side in this volume. The chapters provide the opportunity to compare different approaches towards similar goals in establishing and developing biobanks. I hope this will provide useful models for many other countries trying to set up their own biobanks.

The remaining chapters in the book provide different outlooks on sustainability, cell preservation strategies, growing human biospecimen needs, and personalized medicine. Included in the book also is a chapter reasoning the needs for a strategic focus from a sample dominated perspective to a data-centric strategy (Chap. 13).

I am honored and owe much gratitude to all those who agreed to contribute and assist me in preparing this publication. I am thankful for the support and trust of Springer Publications, in particular Senior Editor Dr. Meran Owen and Tanja Koppejan, and my family for their patience.

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Integration, Networking, and Global Biobanking in the Age of New Biology

1

Feridoun Karimi-Busheri and Aghdass Rasouli-Nia

Abstract

Scientific revolution is changing the world forever. Many new disciplines and fields have emerged with unlimited possibilities and opportunities. Biobanking is one of many that is benefiting from revolutionary milestones in human genome, post-genomic, and computer and bioinformatics discoveries. The storage, management, and analysis of massive clinical and biological data sets cannot be achieved without a global collaboration and networking. At the same time, biobanking is facing many significant challenges that need to be addressed and solved including dealing with an ever increasing complexity of sample storage and retrieval, data management and integration, and establishing common platforms in a global context. The overall picture of the biobanking of the future, however, is promising. Many population-based biobanks have been formed, and more are under development. It is certain that amazing discoveries will emerge from this large-scale method of preserving and accessing human samples. Signs of a healthy collaboration between industry, academy, and government are encouraging.

Keywords

Scientific revolution • New Biology • Population-based biobanking • Harmonization and standardization • Bioinformatics • Global biobanking

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1.1 Introduction

Biological revolution and its implication in health sciences would inevitably become one of the most significant advances in human history in the

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twenty-first century. The explosive advances in biological sciences in latter half of the twentieth century and early twenty-first century have paved the way for remarkable possibilities to be developed by future generations. The discovery of DNA's double helix structure in the early 1950s [1] was a key breakthrough in modern biology and probably one of the most important discoveries in scientific history, that fueled a continual stream of new and fascinating discoveries, tools, and methodologies. Today, almost 70 years later, we are witnessing the contribution and the paramount significance of this massive discovery in every corner of biological, health, and pharmaceutical sciences [2]. Many new branches have emerged in this revolution that have become common words known to junior high school students and through media in every household; including: bioengineering, bioinformatics, biotechnology, molecular biology, nanobiotechnology, and biobanking. This chapter discusses how and why biobanking has transformed to 1 of the 10 emerging ideas changing the world and how the power these repositories provide to researchers and clinicians seems to justify this claim [3].

1.2 Towards the Consensus on a Definition

The definition of biobank is varied and there is no consensus [4]. Traditionally, biobank was considered, and still is, a term defining the biorepository of biological tissues. Biobank, however, is used in a much broader context today, covering not only human tissue storage but population-based and disease-based biorepositories, non-human material, genetic material, specimens of endangered species, and any collectable sample or data that exists in the biological kingdom [5]. Other characteristics of biobank based on purpose, ownership, volunteer group, or size have also been included in the terminology [6, 7]. By this broad definition then biobanking has the potential to unlock many new doors for researchers studying human and non-human population-based collections. Though there are huge technical and ethical challenges that need to be addressed before the full potential of these biorepositories can be realized, the field is advancing rapidly in terms of sample acquisition, storage, and practice to overcome these hurdles.

1.3 The Age of New Biology

It is believed that the twenty-first century will be the "century of biology" [2]. In 2009, at the request of some of the US Federal Departments and Agencies, a committee was set up by the National Research Council's Board on Life Sciences to study the state of biological science in the United States and propose guidelines about biological research in the future in light of the advances technology and science disciplines have brought and how these resulting dimensions have forever changed the way we conduct biological research. The committee members were recruited from diverse disciplines of biology, engineering, and computational sciences. The structure of the committee from day one indicated the importance of integration and interdisciplinary collaboration in the future of biological research. The report made some strategic recommendations and conclusions for a "New Biology for the 21st Century" [8].

The findings of the committee, not surprisingly for scientists and strategists, led to recommendations of some fundamental principles for the future. The report, though prepared for the U.S., could be equally a strategic guideline for the future of biology globally. In summary, the key report highlights pertain to "integration" and "collaboration" of biologists with scientists and engineers. This collaboration has already made it possible to collect vast amounts of data and detailed observations in a fraction of the time possible to reach by human.

Defining the fundamental questions facing future biobanking in the age of a biological revolution, and determining how future biobanking can capitalize on these advances in science and technology are the main challenges ahead of this industry. More global centralization and harmonization should probably be the immediate short term objective of biobanking.

One important goal of New Biology is the approach towards monitoring personalized health that could significantly revolutionize personal and public health medicine [9]. This vision is already the foundation of national population-based biobanks under development around the world, a resource that will stimulate and speed up research and our understanding of predictive, preventative, and personalized medicine.

Biobanking has a historical opportunity to contribute to the understanding of how all of the components of living systems operate together in biological organisms, healthy or non-healthy, which could lead to new approaches to prevent and cure chronic and acute diseases. Future biobanking in the age of New Biology would be a powerful resource to solve many questions regarding the incredible diversity and the dynamic complexity of human beings; including, evolution, human behavior, and cutting edge research on anti-aging and longevity. This is not a futuristic dream considering what biobanking is building even now: a potentially vast amount of data and information that will be stored throughout the biobanks around the world is being generated from DNA, blood, saliva, tissue and tumors, umbilical cord, urine, physical measures, hair, teeth, MRI and other scans [10]. Many of these specimens are ideal samples for genetic, proteomic, epigenetic, metabolomic, and biochemical analysis.

1.4 Integration and Networking

Creation of national biobanks has exponentially increased the size of samples, highlighting the need to replace manual protocols with automation and integrating a wide range of scientific disciplines. Biobanking of the future is not limited to only coping with a few freezers and liquid nitrogen tanks. UK Biobank, as the biggest biorepository and biomedical database in the world, for example, has securely-stored an incredible amount of data of about 20 terabytes, equivalent to approximately 30,000 CDs stacked to a height of 35 m, from the donated blood, urine, and saliva of roughly half a million people during 2006–2010. It is expected that the data base will expand enormously in coming years to also include functional magnetic resonance imaging (fMRI), ultrasound scans, and X-rays of bones and joints [10]. This massive project could never have been started without the integration of a team of engineers, biotechnologists, information technologists, bioinformaticists, epidemiologists, and scientists.

National banks, similar to this project though varying in size, have also been developing in other countries. In the pan-European Biobanking and Biomolecular Resources Research Infrastructure (BBMRI), for example, approximately 52 participating institutions and 200 associated organizations from 30 countries are involved. The mission of BBMRI is the construction of a European biobank for collecting biological samples from healthy and non-healthy individuals, setting-up biocomputing facilities and analysis tools, and establishing an ethical, legal, and social framework [11]. BBMRI's vision for the future is an excellent model for a modern biobank that requires the collective efforts of clinicians, basic scientists, epidemiologists, laboratory medicine experts, 'omics' and technologies experts, database and IT experts and statisticians, and could only be achievable through a federated network of centers by European Union members [12].

1.5 Revolutionary Milestones

1.5.1 Human Genome

The first and final draft of the human genome sequence was published more or less on the fiftieth anniversary of the discovery of DNA [13]. The human genome has an estimated 3.2 billion nucleotides coding for 20,000–25,000 genes [14]. Sequencing of the human genome not only will enhance our understanding of human diseases and treatment but it will also significantly improve our insights into how genes are controlled and function in humans. The project is a paramount achievement in the history of science that could not have been possible without the

academia-government-industry partnerships for the development of new technologies through the combination of large-scale and high-throughput generation of biological data.

In the mid 1980s the best sequencer was able to read 1,000 base pairs a day. In the beginning of 2000, 15 years later, machines could sequence 1,000 base pairs per second, equal to over 86 million base pairs in 24 h [15]. Emerging technologies such as nanopore technology, using electric fields to drive strands of DNA through a small hole, are capable of sequencing an entire human genome for less than $1,000 at a speed of one base per 10 ns [16].

1.5.2 Computers, Bioinformatics, and Common Language

If one were to be asked what the closest partner and most significant part of Biobanking in the twenty-first century will be, then Information Technology would be the right answer. Information technology, in other words, is the foundation on which future biobanking rests. One of the most challenging issues in modern biobanking is the ever increasing complexity of data management and integration [17]. Advanced high throughput profiling machines are constantly adding several hundred gigabytes of data into the biobanks. Biobanks and repositories are no longer limited to one laboratory or department and facility, as heterogeneity of the collected data is so immense that no single standardization could handle the extensive metadata. Globalization, data sharing, and multidisciplinary collaborations form the prominent entity of biobanks. It will also be important for information technology to provide a "flexible and extensible metadata" with consistent vocabulary and terminology useful in large-scale projects and equipped with data integration capabilities in the global biobanking community [17].

As the number of population-based and large-size biobanks is increasing throughout the world, sharing and ability to access metadata across the institutions and nations will inevitably be a major challenge for global biobanking that can only be solved by information technologists. Development of an efficient biobank requires extensive support from software tools. Many different models have already been developed or are under development to be able to handle these mega metadata, such as XTENS [17], i2b2 platform [18, 19], CaTissue [20], and SIMBioMS [21]. XTENS, for example, is a novel data model designed to mimic a digital repository with the general purpose of being capable of handling heterogeneous data in an integrated biobanking scenario [17].

Another example is the Stanford Translational Research Integrated Database Environment project at Stanford University (STRIDE) [22]. This project, originally initiated in 2003, represents an integrated standards-based translational research informatics platform to manage biomedical data in translational research using detailed data provided by the electronic medical record. In addition, STRIDE was later used as a sophisticated tracking system with the ability to locate and track each record of a biospecimen. The STRIDE Virtual Biospecimen Bank maintains an online, searchable record of biospecimen attributes and storage location for a number of biobanks at Stanford including their Bone Marrow Transplant program, Hematology Tissue Bank, and Cancer Center Pathology Core [23, 24]. As of 2009, over 50,000 biospecimens had been stored in this virtual unified biobank. Though STRIDE at the moment is implemented only at Stanford, The Stanford Center for Clinical Informatics does not see any reason why this could not be used in other sites.

There are various bioinformatics platforms searching to provide comprehensive solutions for biobanking capable of integrating very large amounts of highly heterogeneous data from multiple sources. None, however, is comprehensive enough to fulfill the requirements of future biobanking that is increasingly demanding data integration [25]. Global biobanking requires a standard well organized integrated software platform in which the researchers are able to locate both the raw and processed data, and potentially connect research groups globally, while preserving the security, privacy, and ethical issues related

to patients and donors. However, how best to create a common platform and successful open collaboration remains a tremendously challenging task without which progress towards global biobanking would be slowed down.

1.5.3 Post-genomic Era

Biobanks are the richest source of material available in the post-genomic era. An increasing source of data from blood to tissues, DNA, saliva, and other sources has generated a tremendous amount of information that is useful to all aspects of omics, arrays, and next generation sequencing. Included under the term of omics are an astonishing number of new post-genomic fields that can benefit from an ever increasing rich source of data stored and collected in biobanks that includes epigenomics, transcriptomics, proteomics, metabolomics, glycomics, lipidomics, microRNAome, methylome, and more to come [26, 27].

On a more practical approach, efforts are underway to directly take the concept of integration to biobanks and combining large semiquantitative metabolomics data to provide a higher statistical power for biomarker discovery and validation. This approach has been employed in combining lipidomic profiling data from different studies where lipidomic data from three different large biobanks were used using an appropriate measurement design and transfer model [28].

As of the beginning of the century it is estimated that there are over 300 million tissue samples stored around the world [29]. Molecular profiling of biospecimen and tissue samples using the wide range of available omic's technologies will provide deeper knowledge of the human genome and diseases and will significantly improve the effectiveness and efficiency of drug discovery and development.

Probably the main challenges for omics technology are quality and standardization of biospecimens and lack of a universal method for collecting, storing, and processing the samples. This might be the main reason why it is difficult to duplicate results from different sites [26]. There are, however, some guidelines designed to clarify the pre- and post-analytical variables. Biospecimen Reporting for Improved Study Quality is an important step towards a more consistent procedure available for researchers and regulators to harmonize their protocols and reduce variability to achieve evaluation quality [30–32]. After all, this tremendous amount of data will enable us to know how our biological system functions or how it fails [33].

1.5.4 Advances in Technical Storage

Ensuring the samples, especially solid tissue samples from medical procedures, are of sufficient quality poses perhaps the most difficult problem as the time from excision to preservation can vary immensely and the state of the cells can vary significantly with time. The stress of excision combined with a change in temperature can induce changes that may alter the tissue properties significantly. In addition to taking measures which ensure high quality primary samples, tests must be developed to determine whether the samples are actually of sufficient quality for use in experiments. Increasing the quality of preservation is of paramount importance to the biobanking industry, as this is the basis for building effective tissue repositories that can be drawn on at any time. A review of 125 biomarker discovery articles published in open-accessed journals between 2004 and 2009 reveals that in more than 50 % of the papers there is no citation of how the biospecimens were obtained and how they were processed [34].

Over the last few decades there have been many different protocols and processes used to collect, store, and analyze biological materials in biobanks. The most commonly used processes of preservation are hypothermic or chemical environment. Though both procedures are considered in one way or another to be the best methods for preservation of biomolecules, both have insufficiencies that have hampered the quality of the biospecimens and the outcome of the results [31, 35–39]. It is not surprising, then, to see that according to a survey by NCI in 2011 nearly half

of the cancer scientists that responded have difficulty in finding high quality samples, which led 80 % of them to limit their scope of research. Sixty percent of these researchers even question the validity of their own results [40].

A number of issues remain to be dealt with to improve the quality, reproducibility, and validity of the results. Common fixative or physical techniques such as formalin, alcohol, acetic acid, heating, microwaving, freeze-drying, etc., all in one way or another affect the integrity of DNA, RNA through degradation, or result in hypoxia or dephosphorylation [41, 42]. Different protocols and innovative procedures have been developed to overcome these problems, such as modification or removing formalin fixation or slowing down processes such as hydrolysis and oxidation by removing water and reactive oxygen-containing molecules [43].

Until recently, less attention has been paid to the investment in and development of storage and preservation techniques. There are essentially two reasons behind this: (i) more complicated analytical and processing technologies such as genome project, omics, and bioinformatics have dominated the field that absorbs the main bulk, if not all, of the fundings, and (ii) shortage of supply and funding for infrastructure. Unfortunately, little effort has been made by researchers to publicize or address these issues.

Fortunately there is increasing interest in overcoming shortfalls in both preservation processes and adequate infrastructure funding to meet the demands of today's labs and clinics. There are improved operational designs for sample storage, annotation, automation, logistics necessary to ship the specimen in ultra-cold condition, and data management software [44].

1.6 Harmonization and Standardization Is the Key

There is extensive effort across North America and Europe to find ways to better coordinate, harmonize, and more consistently standardize information on the collection, access, and research activities in biospecimen and epidemiological data, issues that surprisingly have come under scrutiny only recently. And still there are significant gaps in harmonization of biobanking practices. [32, 43, 45–47].

In 2005 the US International Society of Biological and Environmental Repositories published its first edition of best practice and later in 2010 a coding system entitled Standard PREanalytical Code was introduced [43]. A committee of experts including clinicians, pathologists, laboratory scientists, biobankers, and statisticians set a series of concrete recommendations for authors for biospecimen reporting to improve study quality (BRISQ) [31, 45].

Similarly, the European Union built a consortium of 7 public research organisations, 8 research companies and an official European standards organisation for creation of guidelines and tools to develop quality guidelines for molecular in vitro diagnostics and to standardize the pre-analytical workflow (CORDIS) [48]. According to the first report released by the SPIDIA (Standardisation and improvement of generic pre-analytical tools and procedures for in vitro diagnostics) the consortium launched two successful large "ring" trials on pre-clinical variation on DNA and RNA analysis in 250 laboratories. The consortium has also developed new tissue collection and stabilisation technology, identified biomarkers to monitor changes in clinical samples, and started development of an integrated sample tracking system.

In addition to large agencies in the USA and Europe, there are many other small to medium biobanks and disease specific biorepositories around the world that, in line with the necessity to standardize their protocols have started to develop their own guidelines. Shanghai Clinical Research Center, for example, is using the International Organization for Standardization concept as a model in their biobanking quality management systems [49]. Many other efforts are underway throughout the world, such as National Biobank of Korea, Jordan Biobank, or even smaller countries like Kazakhstan, to standardize their biobanking system [50, 51].

The Government of the Netherlands in 2007 specifically initiated and funded a strategic effort to establish infrastructure for disease-based biobanking called String of Pearls Initiative program [52]. A similar initiative has also been made by the French chronic kidney disease-renal epidemiology and information network [53]. Standardized protocols and uniform consensus definition for cerebrospinal fluid, nonsurgical clinical and epidemiologic data relevant to endometriosis research, chronic kidney disease, and harmonization of pathology quality assurance methods have also attracted intensive international academic and industry collaboration [52, 54–57].

1.7 Financial Prospects of Biobanking

Though the biobanking industry is facing significant challenges in its early days, the value of these repositories is certainly being recognized. A 2009 article in *TIME* Magazine listed Biobanking as 1 of the 10 ideas changing the world right now, and the power these repositories would provide to researchers and clinicians seems to justify this claim [58]. In the United States, the biobanking industry is in its infancy, but the estimated value was still around $8 billion in 2009, and is expected to reach about $19 billion in 2015, and estimated to reach $45 billion by 2025 [59]. The industry is being driven by massive investment from pharmaceutical and biotechnology companies, and is quickly expanding to facilitate stem cell research and personalized medical treatments. Other arenas like the European biobanking industry are slightly better developed due to collective initiatives like the European Biobanking and Biomolecular Resources Research Infrastructure, which is on track to become part of the European Research Infrastructure Consortium and includes over 30 countries and 225 organizations.

The funding and infrastructure challenges of biobanks may also be compensated by the increasing interest of industry to invest in this area and commercial biobanks, which could address the burden of expanding biobanks by offering more economical and innovative both in-house or off-site alternatives [37]. Parallel to so called commercialized biobanks, academic and non-profit organizations are also increasing their efforts to find resources of funding and awarding grants for developing innovative storage technology [43, 60].

Overall, the financial prospects of biobanking in twenty-first Century look solid and strong enough to meet future demands and global networking and expansions.

1.8 Conclusion

Biobanking is going to be the largest ever library of biological materials. At the age of New Biology the major challenge for this mega library is not only the information technology or highly advanced biotechnology but also the standardization of billions of specimens and huge datasets throughout the biobanking world; from large population-based to smaller biobanks spread over many places.

Biobanks should maintain their current policy of not being directly involved in conducting research. Becoming more efficient and collaborative should remain a strategic policy for biobanking. At the same time, biobanks should audit the outcome of the research using their materials by those who have had access to them.

An ideal twenty-first century biobank would be a collection of banks across the world that function as a single entity and not as isolated centers for storing massive data sets. Despite the challenges, as the climate for innovation in the biobanking industry continues to flourish globally, it is certain that amazing discoveries will emerge from this large-scale method of preserving and accessing human samples.

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The Future of Biobanking: A Conceptual Look at How Biobanks Can Respond to the Growing Human Biospecimen Needs of Researchers

2

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Abstract

Biobanking of human biological specimens has evolved from the simple private collection of often poorly annotated residual clinical specimens, to well annotated and organized collections setup by commercial and not-for-profit organizations. The activities of biobanks is now the focus of international and government agencies in recognition of the need to adopt best practices and provide scientific, ethical and legal guidelines for the industry. The demand for more, high quality and clinically annotated biospecimens will increase, primarily due to the unprecedented level of genomic, post genomic and personalized medicine research activities going on. Demand for more biospecimens provides new challenges and opportunities for developing strategies to build biobanking into a business that is better able to supply the biospecimen needs of the future. A paradigm shift is required particularly in organization and funding, as well as in how and where biospecimens are collected, stored and distributed. New collection sites, organized as Research Ready Hospitals (RRHs) and new public-private partnership models are needed for sustainability and increased biospecimen availability. Biobanks will need to adopt industry-wide standard operating procedures, better and "non-destructive" methods for quality assessment, less expensive methods for sample storage/distribution, and objective methods to manage scarce biospecimens. Ultimately, the success of future biobanks will rely greatly on the success of public-private partnerships, number and diversity of available biospecimens, cost management and the realization that an effective biobank is one that provides high quality and affordable biospecimens to drive research that leads to better health and quality of life for all.

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Keywords

Biobanking • Biospecimen • Biobank networks • National biobanks • Regional biobanks • Community hospitals • Academic biobanks • Research ready hospitals • Sustainable biobanking

Abbreviations

BBRB	Biorepositories and Biospecimen Research Branch
CAP	College of American Pathologists
CBCP	Clinical Breast Care Project
CDP	Cancer Diagnosis Program
CHTN	Cooperative Human Tissue Network
CRO	Contract Research Organization
ISBER	International Society for Biological and Environmental Repositories
NCI	National Cancer Institute
RRH	Research Ready Hospitals
SOPs	Standard Operating Procedures
TCGA	The Cancer Genome Atlas

2.1 Introduction

Biobanking involves the systematic procurement, processing, annotation, storage and distribution of biospecimens for research activities. Biobanking of human specimens is now an important activity in many institutions as part of a broader strategy to support and advance high impact biomedical research. The majority of human biological specimen collection and storage for research originated from the collections of researchers who had access to patient populations and took advantage of the availability of "left over" specimens to initiate private collections for their immediate or future use. With time, and as the need and value for such research materials continued to gain popularity, especially driven by the "genomic and post genomic revolution", the need for more coordinated and comprehensive collections became apparent. This gave rise to expanded biobanking efforts initiated by academia, government, private and commercial entities with the aim of supporting a broader research base.

Many research programs have benefited from specimens provided by biobanks. For example, the development of the antibody trastuzumab known as Herceptin [1, 2] which is used for treatment of a specific sub-population of breast cancer patients relied on the evaluation of tumor specimens stored at the National Cancer Institute's Cooperative Breast Cancer Tissue Resource. While advances have been made through the use of biospecimens from biobanks such as these, there continues to be the need for more biospecimens to meet the overarching need of scientific discoveries as it expands to rare diseases, cancer disparities [3] and basic research which provides the focus and direction for translational research. As the value of this critical resource increases so will be the need to increase supply and standardize the process of consenting, sample collection, storage and quality assessment.

The biobanking industry will continue to evolve, and the next 5–10 years will see the emergence of biobanks that are more sophisticated in design, expensive in operation and closely regulated to maintain quality and prevent illegal and unethical practices. The changes expected in the industry will mainly be recommended or mandated by government, specialized organizations such as the International Society for Biological and Environmental Repositories (ISBER) which promotes the development of best practices and guides the future direction of the industry as well

as reputable organizations like the College of American Pathologists (CAP) which has partnered with ISBER to create an accreditation pathway for biobanks to ensure similar standards across the industry. With accreditation comes the expectation of a certain level of quality for biospecimens from accredited biobank.

As the demand for good quality and well annotated tissue increases, the cost of biospecimen acquisition is expected to correspondingly rise especially in developed countries where most of the advanced biomedical research occurs, and the value of good quality and well-annotated specimens are fully appreciated. The increase in cost will primarily be due to market forces associated with scarcity of good quality and rare specimens, and the added cost of maintaining high standards and best practices mandated or recommended by organizations like ISBER and CAP. To keep cost under control while increasing biospecimen availability and quality, biobanks in developed countries, such as the United States will need to re-think existing methods and strategies for biobanking. New strategies will have to be developed to gain access to more donors while still maintaining (a) the biobanking standards that will ensure availability of high quality biospecimen and (b) a cost structure that is affordable not just to big pharmaceutical industries and well-funded projects that can pay for quality and faster turnaround time, but also to other biomedical research scientists in academia and small businesses who typically have limited budgets. This chapter discusses the biobanking industry as it is today, and describes the future direction and certain concepts that could help increase biospecimen availability in an inexpensive and sustainable manner.

2.2 The Biobanking Industry Today

Early biobanks were simply the private collections of often poorly annotated residual clinical samples ("left over") collected, maintained and used by a single or small number of scientists. There are many limitations associated with the use of such collections, including inappropriate patient consenting (or in some circumstances no patient consent), inappropriate or no pathological diagnosis, miss-labelling, poor cataloging, thus rendering the specimens inadequate for any serious research. The identification of these impeding issues that hinder the use of legacy biospecimens collected and stored in an inappropriate manner led to the establishment of newer and more organized biobanks, and institutional biobanks which took advantage of university based hospitals to collect, store and distribute biospecimens for various research activities within the university.

Biobanks have since evolved to the extent that many hypothesis generating or hypothesis driven research are made possible by biobanks. As the value of biospecimen resource increased so did the need to increase supply and standardize the process of consenting, sample collection, storage and quality assessment. A number of developed and developing countries now have biobanks specifically setup to support and promote biomedical research as well as a national security strategy. An online directory (Specimen Central, www.specimencentral.com) indicates that there are about 25 biobanks in Asia, 13 in Australia, 80 in Europe, 4 in the Middle East, 13 in Canada and 151 in the United States. The list of biobanks includes university based, commercial (for-profit) and nonprofit private biobanks (Table 2.1). Some of these biobanks are well organized and funded and in some cases they consist of networks where collections from numerous sites are managed at a single centralized resource center, or made available through a virtual database. These biobanks cover a wide variety of disease areas with some focusing on specific disease conditions and others capturing a wider variety of health conditions.

As the need grew to understand the molecular and environmental basis of diseases with research aiming to improve diagnosis and treatment, the activities of biobanks have become a focus for government agencies such as the Biorepositories and Biospecimen Research Branch (BBRB – http://biospecimens.cancer.gov/default.asp) of the Cancer Diagnosis Program (CDP) of the United States National Institutes of Health. This national initiative which was established to systematically

Table 2.1 Biobanks per country with their classification

Country	Total	University	Commercial	Non-profit	Networked[a]
Austria	3	2	1	0	0
Belgium	2	1	0	1	0
Switzerland	5	1	3	1	3
Cyprus	1	0	1	0	1
Germany	5	0	0	5	4
Estonia	1	1	0	0	0
Spain	4	0	0	4	2
Finland	2	0	0	2	1
France	6	0	0	6	3
Greece	1	0	1	0	0
Hungary	1	0	0	1	1
Ireland	5	2	1	2	1
Italy	6	0	1	5	4
Latvia	1	0	0	1	1
Netherlands	3	1	0	2	0
Norway	2	2	0	0	0
Sweden	5	2	0	3	1
UK	25	17	3	5	0
Canada	13	5	0	8	2
United States	151	65	29	57	6
China	3	1	1	1	0
India	5	1	1	3	0
Japan	4	1	0	3	0
Korea	2	0	1	1	0
Malaysia	3	1	1	1	0
Singapore	3	1	1	1	0
Taiwan	1	0	0	1	0
Thailand	2	0	0	2	1
Australia[b]	13	2	0	11	1
Iran	1	0	0	1	0
Israel	3	1	1	1	0
Total	**282**	**107**	**46**	**129**	**32**

Data adapted from www.specimencentral.com

[a]Indicates how many of the biobanks are made up of Biobanking networks. This number is not part of the Total

[b]Australia though listed under Country, is a Continent

address and resolve the problem of biospecimen collection, is aimed at providing guidance for the acquisition of very high-quality human biospecimen. The aim of BBRB is to facilitate biomedical research through improved quality standards for biorepositories. In Europe, the Confederation of Cancer Biobanks provided guiding principles applicable to the management, operations, ethical and legal environment of human biospecimen resources for the United Kingdom from 2006 onwards. The list of government's effort and their involvement in the proper and efficient operation of biobanks continues to grow, and cuts across countries. The ultimate aim of this involvement is to ensure that the best ethical standards and methods are used to acquire sufficient specimens from consented donors to boost biomedical research. This includes provision of the variety of biospecimen required for different research activities and ensuring that high quality standards are maintained

to produce research results that lead to a better understanding of diseases development and progression, and provide new treatment options.

One of the most highly organized national biobanks in operation is the Cooperative Human Tissue Network (CHTN) of the Cancer Diagnosis program of the National Cancer Institute (NCI). The CHTN was established in 1987 [4] to provide increased access to human tissue for scientists in academia and industry in the areas of basic, applied and translational research. Today it remains one of the oldest well organized human tissue resources. The NCI has also funded various other biobanking efforts in a number of academic institutions to supply the tissue needs of specific research programs. For example, the Western Pennsylvania Genito-Urinary Tissue Bank located at the University of Pittsburgh was established in 1991 to collect and bank tissue from a cluster of hospitals. This program quickly expanded into other sites including George Washington University, Washington, District of Columbia and the Medical College of Wisconsin, Milwaukee. Through such a program, thousands of prostate samples have been made available to researchers, together with serum, whole blood, lymphocytes and associated clinical data. While biobanks have been in existence for over 60 years [5] it was only in the last 20 years that they expanded in complexity and utility [6]. Reasons for their establishment have ranged from the need to intentionally address particular research needs, or perceived opportunity/expectation. To date the industry is still dealing with a number of issues including that of classification [6, 7]. In 2003 RAND Corporation classified biobanks into government, academia, and industry but the report provides little information on the organization of biobanks, how they function and their potential for long term sustainability [8]. A 2012 national survey of biobanks in the United States shows great diversity in the organization and operation of biobanks [7].

The business opportunity and demand for more and higher quality biospecimens continues to drive developments in this industry. While research scientists who had access to patients and "left over" tissue started the early biobanks, the biobanks of the future will be more sophisticated and receive startup and operating funds from government, large companies and venture capitalists. The global biobank market is expected to reach over US$22.3 billion by the year 2017 [9], driven mainly by political, scientific and business interests, enhanced government and private funding, as well as greater public awareness and support.

The United States and Europe currently dominate the biobanking market in terms of sheer size. It is however predicted that in the long term, many more biobanks will develop in the rapidly burgeoning and heavily populated markets in Asia, Latin America and emerging markets to support the increasing demand for biospecimens in USA and Europe. This prediction is already manifesting because in recent years major pharmaceutical companies have started sourcing for biospecimens from countries such as China [10]. The driving force behind this move to countries like China is partly due to easier access to patient populations and significantly lower cost of biospecimen acquisition. This scenario has the potential to reduce the quality of specimens supplied due to lesser regulation and quality control practices outside the United States and Europe. Also, the cost of biospecimen acquisition in developed countries will continue to increase since only a small number of biobanks will remain operational if most people that need specimens get them from foreign suppliers. An increase in acquisition cost will drive this essential resource out of the reach of small and medium sized companies thereby hindering research.

2.3 The Future of Biobanks

Biobanking has a bright future as a business and a critical resource for translational research which will move research from the bench to the bedside. The future of biobanks will be shaped by many factors including how biobanks respond to the expected increase in (a) demand for more and different types of tissue and accompanying information, (b) regulation, (c) sophistication and (d) operating expense. As the value of good quality

and well-annotated human biospecimens is increasingly appreciated by more researchers especially in developed countries, the number and type of tissue available for research will have to increase without a significant increase in the cost of acquisition and distribution. Biobanks will become more of a business than a "hobby" and unaccredited banks and those that cannot provide quality tissue, or operate in an efficient and sustainable manner will be unable to compete and stay in business.

It is expected that core research programs and government agencies will become more involved in biobanking either by setting up programs to support in-house research or fund existing biobanks to collect and bank tissue for their research. A good and successful model is the disease/research focused program of the Clinical Breast Care Project (CBCP), a Department of Defense funded collaborative effort between the Walter Reed National Military Medical Center, Windber Medical Center and Windber Research Institute. This program which was setup in 2001 has a multidisciplinary approach to breast disease research, integrating clinical (prevention, screening, diagnosis, treatment, risk reduction), translational research, biobanking and informatics. The CBCP biobank is located at the Windber Research Institute, Windber PA. The biobank acquires and stores good quality, well annotated human breast tissue from collaborating medical centers/clinics of the CBCP program. In this way, researchers within the program have adequate supply of extensively annotated biospecimens for their genomics, proteomics and cell biology research activities without the hassles of looking for suppliers. The CBCP tissue bank has since evolved to the extent that it now banks tissue for other organizations, and it is currently being audited by CAP for accreditation.

The future will also see the setting up of more generalized biobanks such as the Cooperative Human Tissue Network (CHTN) that collects every available specimen to support every possible type of biomedical research. Generalized biobanks like the CHTN are essential because not all research programs will have sufficient capital and trained manpower like the CBCP program to maintain a fully functional biobank. Biobanks of the future will continue to strive to have sufficient number and type of good quality biospecimens required by the research community it serves. But experience teaches us that even the largest and well organized biobanks do not always have the specific type and number of biospecimens required by every researcher. To support the expected increase in demand for human specimens for research, biobanks will have to re-think the methods used for donor enrolment, specimen collection, storage and distribution to researchers.

The genomic and post-genomic revolution will continue to influence the number and type of tissue required for research in the future. This revolution has seen researchers looking far and wide for human biological material to perform genomic and proteomic research. The demand for human biological material will continue to increase and researchers requiring these specimens for molecular profiling to identify diagnostic markers and therapeutic targets will increase. This phenomena already resulted to the development of more coordinated collections to feed specific research needs, and the advent of disease centric, genetic, population or "bioproduct-based" (example, DNA) biobanks [11]. In the past, academic institutions with medical schools responded by establishing biobanking core facilities which fed the research needs of their scientists. These scientists were now able to procure specimens at minimal cost as the institution absorbed most, if not all, of the overhead associated with procurement, processing and storage. With more funding from governments, Department of Defense and numerous other funding agencies, more biobanks will be setup to feed the research needs of the future.

All biospecimens will be affected by different collection, processing, shipping and storage conditions, leading to small, medium or extensive changes in their molecular composition and quality. Small to moderate ex-vivo changes may not render a biospecimen completely useless. But this must be recognized, documented and taken into consideration when using the biospecimen. Thus, as biobanks evolve and become more

sophisticated, it will be necessary to adopt a universal method for determining and reporting biospecimen quality. To ensure that the quality of biospecimens obtained from different biobanks can be compared, it is critical that information regarding the handling of biospecimens be reported in a thorough, accurate, and standardized manner. Such standardized method for evaluating biospecimens is provided through "Biospecimen Reporting for Improved Study Quality (BRISQ)" [12]. Using BRISQ for biospecimen evaluation provides end users and stakeholders with more consistent and standardized information to better evaluate, interpret, compare, and reproduce their experimental results which utilize biospecimens.

Another important issue that biobanks in the future will have to deal with is how the quality of a biospecimen is precisely determined. Presently the quality of RNA extracted from tissue is commonly used as the basis of determining the quality of the tissue. But RNA based methods may be destructive and results obtained may not be ideal for all circumstances. For example, samples demonstrating degraded or partially degraded RNA may still be useful for some studies. Moreover, it may not be possible to isolate and analyze RNA from every biospecimen. Although no single method may be found that is universally suitable for all biospecimens, biobanks of the future will need to develop alternate or complementary methods, including nondestructive methods that can be used to assess and establish biospecimen quality at the molecular level. An ideal method should not destroy or exhaust the biospecimen in the process, the data obtained should be relevant at the DNA, RNA and protein levels and it should be easy and inexpensive to perform.

2.4 Increasing Biospecimen Supply

The need to increase biospecimen supply has been recognized for some time. Increasing biospecimen supply has been approached in different ways including setting up of commercial biobanks, national biobanks and biospecimen networks. Irrespective of the organizational and business structure of the biobank, it is expected to serve as a resource to support and advance biomedical research. Human tissue donated and stored should be available to scientists in academia, and the business/private sectors (pharmaceutical and biotechnology industries). Whether biobanks are set up as nonprofit or for-profit, the ultimate goal is that their collection of human tissue specimens will be utilized for research leading to diagnostic, therapeutic and predictive clinical products.

To effectively support basic and translational biomedical research in a sustainable manner, biobanks must obtain appropriate consent from donors, acquire good quality specimens, maintain these specimen in storage conditions that retain the tissue integrity and make these specimens available for research on demand. A biobank that does not distribute its biospecimen effectively to researchers would not be contributing to the general goal and aims of biobanks. On the other hand, a biobank could fail to have biospecimens for distribution due to unavailability of the specific type of biospecimen in demand or inadequate supply of donated material. These are important issues that are still inadequately addressed in the biobanking industry. Scientists will need to have access to inexpensive and relevant biospecimens in order to move their research activities forward, and this will be made possible by commercial, national and new generation biobanks established in response to the demand for biospecimens.

2.4.1 Commercial Biobanks

Commercial and business driven biobanks will play critical roles in biospecimen availability. In the early 2000s, a number of commercial biobanks, such as Ardais, Asterand, Genomics Collaborative, were set up to fill the unmet needs of researchers both in academia and the pharmaceutical industry. Typically, the commercial banks enter into an agreement with hospitals from where the specimen is collected and the specimen is either banked or supplied directly to

the end user. Because such banks are commercially driven, they are more business oriented, are not limited by institutional or national boundaries so are better able to acquire the number and type of samples required by a researcher faster. Unfortunately, the cost of biospecimen procurement by the commercial companies is often significantly much higher than that of a university based biobank. As a consequence, researchers without adequate funding therefore could not patronize these commercial options. Such a situation and the down trend of the global economy saw a number of the commercial biobanks folding up. The current trend of biospecimen acquisition from commercial companies that is adopted by organizations such as pharmaceutical industries is to employ commercial Contract Research Organizations (CRO) with appropriate expertise in global comprehensive sample and data management to source for biospecimens in countries such as China where a strong environment for clinical research is developing [10]. These are definitely options outside the reach of researchers in academia, start up biotech industries and private research institutions.

2.4.2 National and Regional Biobanks

A welcome development is the establishment of biobanks that will provide different types of biospecimens at the national level. Such scale of biobanking will be needed as many more researchers look for more specimens with better clinical annotations. A number of countries including, Iceland, Sweden, Singapore, the United Kingdom, Croatia to name a few, have already established nationally delimited, population-based or genetic-based biobanks [11, 13, 14]. Some of these banks are public/private partnerships, and funding is from various sources including venture capitalist; charities, pharmaceutical companies and national research council's [13]. These national banks operate as not-for-profit or for-profit entities. The national population based biobanks are well-positioned to provide a wide variety of specimens that help researchers address the complexity of biological processes through systems biology and to analyze other complex contributions to diseases such as genetic and gene-environment interactions [13, 14]. National biobanks represent contributions from thousands of donors and thus they provide researchers sufficient number of biospecimens and hence the statistical power necessary to identify the relatively weak contribution of clusters of small genetic polymorphisms to disease and their effects on risk factors and drug action [13]. It is expected that more national biobanks fully or partially funded by government will be established in many more countries as a cost-control strategy to support and promote translational research of national interest.

2.4.3 Biobanking Networks

Collection and consolidation of biospecimens from multiple sites will be relevant especially when it is not possible to setup a biobank at the national scale. Today, basic and translational research focused on molecular biological techniques, genomics/gene profiling, biomarker profiling, proteomics require freshly donated tissue specimens. Unfortunately, the goal of meeting all research needs is hardly fully achieved at all times by a single biobank. Thus, biobanking strategies which utilize multiple collection sites acting as networks of tissue sources as observed in Europe and the United States [15–19] will have to be established particularly in academic institutions with medical centers and integrated pathological services [20]. Indeed, biobanks have already developed strategies for combining biospecimen collections from a network of collection sites as a way to increase the number and types of specimens available to many more researchers for research. The success of such an approach has led to the establishment of national networks like the pan Europe Biobank Network [18, 21]. Two main network models exist, the Centralized Model where samples are transferred from peripheral sites to a central biobank for processing and storage and the Federated Model where samples are stored at peripheral collection

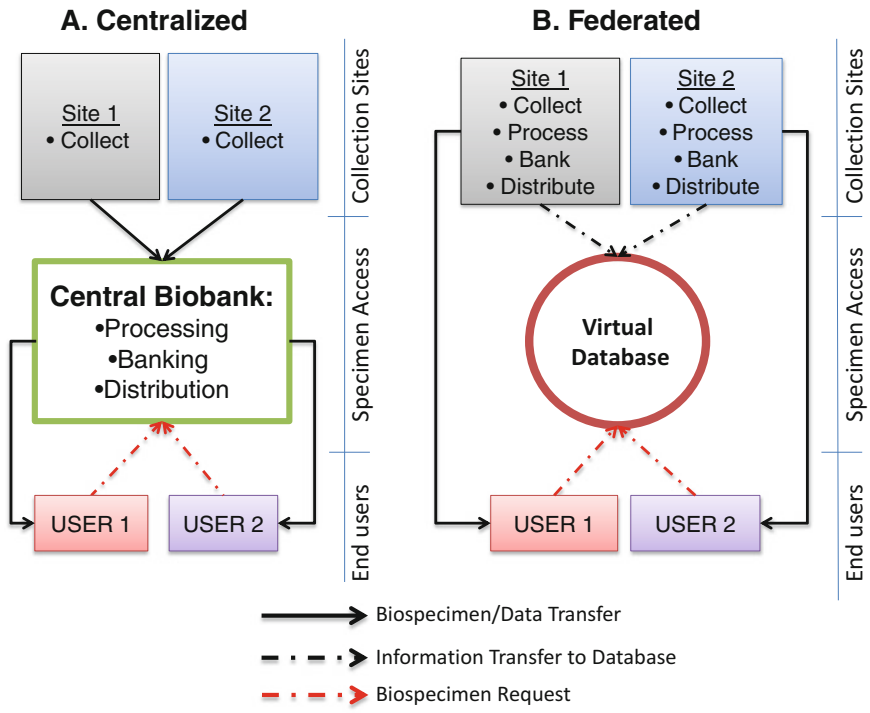

Fig. 2.1 Centralized and Federated Models of Biobanks: (**a**) The Centralized model operates by a network of collection sites feeding a central biobank with samples obtained from donors. The central biobank maintains these biospecimens and distributes them appropriately to researchers (end users). (**b**) For the Federated model, a number of independent sites collect; process and store biospecimens. The information associated with the specimens are made available on a virtual database which can be accessed by the researchers. Request for specimens are made through this central database and appropriate sites distribute the required specimens to end users

sites until needed [22] (Fig. 2.1). These collections are combined virtually by uploading accompanying sample information onto a central database [22]. In this way researchers can identify collections of interest and access them from the multiple collection sites. Such large banking initiatives which utilize network of collection sites at the national scale to increase banking capacity will be an alternate way forward to feed the growing needs of biomedical research.

Researchers associated with biobank networks will be the beneficiaries of these biospecimens and those without such affiliations will be left to source for biospecimen for research from other sources, including commercial companies. But the establishment of biobanks and biobank networks only in academic institutions to supply academic researchers with specimens leaves nonacademic researchers, private research institutions, biotechnology companies and pharmaceutical industries without a unique source of biospecimen. This means they will have to rely on commercial biobanks that are generally more expensive to work with. The Cooperative Human Tissue Network (CHTN) of the National Cancer Institute recognizes the need to work with researchers from academic and non-academic institutions without medical centers and makes CHTN tissue specimens available from their network to these groups of researchers [4]. The biobanking industry will need more innovative models if a good percentage of the biospecimen needs of researchers are to be met.

2.4.4 New Sources of Biospecimens

A strategy that would increase the amount of biospecimens available for research is to obtain samples from nontraditional sources. For example,

there has been growing interest to source biospecimens from hospitals outside the academic setting [20, 23, 24], especially from regional and community hospitals. Since a great number of patients are also seen and treated at Regional and Community hospitals, these establishments can serve as additional avenues to capture patient donors outside the traditional academic hospitals and clinics. Moreover, since most academic institutions are located in cities they typically have samples from urban populations. The integration of community and regional hospitals into biobank networks will be beneficial and help increase the available biospecimen resources with the added benefit of providing a more diversified population of donors and disease categories. The location of these community hospitals provides wider population coverage across states and regional boundaries which include remote, underserved, minority and diverse populations not fully represented in the collections of major academic institutions and hospitals in large urban areas.

2.5 Biobanking Strategies of the Future

The overarching reason for promoting the establishment of new biobanking strategies is to make biospecimen available on demand and at an affordable cost. The strategy should;

(a) Make available larger biospecimen numbers.
(b) Increase the variety of biospecimens available for research.
(c) Increase the availability of biospecimen representing rare disease conditions.
(d) Increase the number of procurement centers.
(e) Provide specimens in a sustainable manner.

The success of a biobanking paradigm shift will require rethinking of processes and methods of operation, the establishment of community and regional hospital networks to source for biospecimens and modifying the way biobanks collect, store and distribute specimens. Developing an integrated biobanking program that is a hybrid of the "centralized" and "federated" models would create the perfect environment for increasing biospecimen supply while standardizing and centralizing banking activities such as design, management, and implementation of standard operating procedures, staff training and database structure. This has been exemplified in the operations of the CHTN which has a Coordinating Committee that oversees the operations of the network to assure quality control and efficiency of network operations [4]. Also, it is important to note that CHTN provides rare and important pediatric specimens which feed researchers from all regions of the network.

The speed with which basic research is translated from the bench to the bedside will depend a great deal on the availability of good quality and clinically annotated biospecimens [25]. As medicine moves towards a more personalized approach, there is bound to be an increasing dependence on high quality biospecimens for medical research. Thus biobanking will play an even more significant role in how medicine is practiced in the future. The success of personalized medicine will therefore partly depend on how available and accessible biospecimens are to the scientific community as a whole. The standards maintained, its sustainability and general harmonization of activities will be key to success. Biobanks will need to be organized in a way that allows them to work together as separate but collaborating entities. Also, the source of biospecimen, which is the hospitals, need to be expanded outside the academic institutions to include other hospitals such as community based hospitals. Such an approach will expand the opportunity for gathering the needed biobank resources – the biospecimens. A number of researchers will need large sample numbers to provide appropriate statistical power for their experiments. Currently, researchers have problems meeting such standards except studies designed as institutional collaborations, such as The Cancer Genome Atlas project (TCGA), a joint effort by the National Cancer Institute and the National Human Genome Research Institute (http://cancergenome.nih.gov/abouttcga). This

project has utilized over 500 samples from multiple sites consisting of academic/nonacademic institutions and medical centers for a single focused research project.

A strategic approach that leverages the "centralized" and "federated" biobanking models will be ideal. A conceptual model is the setting up of a network of Research Ready Hospitals (RRH) that will be easy-to-work with and ready-to (a) collect and bank or (b) collect and distribute on short notice. The RRH concept will require a central coordinating or organizing body to train selected staff at the RRH. The central body will provide resources to ensure that normal hospital functions are not compromised, utilize resources already available in the hospitals, integrate existing hospital regulatory standards with current biobanking standards and streamline collection and distribution protocols that improve efficiency and reduce waste. The sustainability of this system will be built on, among other things, the knowledge and determination of stakeholders to be part of the effort to provide quality biospecimen for research, and contribute to improved health for all while being reasonably reimbursed.

2.5.1 The Research Ready Hospital Concept

The need to develop new strategies to increase biospecimen availability, reduce cost of acquisition and increase availability of a wider variety of biospecimens, especially as it pertains to rare and unique diseases and to cover more racial/ethnic diverse populations cannot be over emphasized. The existence of less racially/ethnically diverse biospecimen resources will limit the application of research findings especially as scientists "pool" existing specimen collections from multiple sites to increase sample size and statistical power [3]. The concept is that the Research Ready Hospitals will have all resources in place in member hospitals such that they will be ready and able to acquire biospecimens from donors when needed, without compromising normal hospital functions. Research Ready Hospitals (RRH) will:

(a) Have all administrative, management, technical and regulatory requirements completed in advance.
(b) Be able to provide multiple types of biospecimens.
(c) Be able to store specimens for short periods of time if needed.
(d) Be able to ship specimens to end users or to a centralized biobank for long term storage.

A project of this nature will be more successful if it is a public-private partnership that will include local communities, hospitals and the coordinating business entity. The RRH concept will empower stakeholders to participate and contribute to research and increase capacity building. While these partnerships will be the key to success, the constraints to such relationships are known and include the lack of a full understanding of the economics of biobanking initiatives, understanding the true market need for biobanks, poor strategies for sample distribution and infrastructure development, management/administration [26, 27]. Potential improvements to these constraints will bother around the areas of providing quality assurance, quality procedures, scientific and clinic-pathological expertise and strict governance for project management by well-established biobanks from both academic and non-academia [27]. On the other hand, the RRH hospitals must be viewed as partners not just biospecimen suppliers with well-defined intellectual property rights if and when applicable. Both parties will also need to understand and define individual cost contributions with legal documentation to protect each party [27].

The amount of work and resources required to get hospitals ready, will vary and be hospital dependent. A typical community hospital may not have an integrated pathology unit, trained personnel, relevant equipment and other unique resources needed to operate a biobank. To address such issues, the proposed partnership will consider the following – the RRH's biobanking

activities would be designed and overseen by an established and accredited biobanking organization (academic/non-academic) that will manage all technical and operational activities related to biobanking and will ensure biobanking standards and best practices are maintained in these RRH. Since the College of American Pathologist (CAP), the same body that provides hospital accreditations in the United States, will accredit biobanks, hospitals will already be familiar with the accreditation requirements and process. All hospitals in the RRH network will be accredited to make them compliant and relevant. The accreditation process for the RRHs and the coordination and supervision by accredited biobanks will ensure the use of best practices. All required standards necessary for ethical and quality biobanking will be maintained by the hospitals. Staff performing biobanking activities will undergo training by qualified staff of the coordinating biobank and all appropriate Standard Operating Procedures (SOPs) will be provided to each RRH. In this way, the hospitals will be well prepared to collect specimens unique to their specialties, or based on specific requirements/demand. Additionally, these RRHs will also be able to identify (based on their training) disease conditions that are unique and potentially classified as "rare" conditions as these will need to be captured as they become available.

The coordinating biobank will maintain communication with the researcher and coordinate sample collection on behalf of the researcher through the RRH. The described RRH concept should be easy to implement because it has some of the attributes already implemented by existing institutional/national biobanking networks such as the CHTN. However, the uniqueness of the RRH option will include; (a) making the RRH hospital a partner rather than just a supplier, (b) building a biobanking enabling infrastructure and awareness at the RRH that covers management, administration and technical staff and not just the pathology department and (c) operating a hybrid "federated" and "centralized" model that is flexible and able to "collect and distribute" as well as to "collect and bank". The RRH hospital will be capable of implementing two workflow processes; which are, acquiring biospecimens, processing and supplying directly to the researcher or acquiring biospecimens, processing and sending to the coordinating biobank for storage and distribution to the researchers as necessary (Fig. 2.2). It is possible that due to proximity of a RRH to the coordinating biobank, fresh (unprocessed) specimen could be transported immediately to the coordinating bank for processing, distribution and storage.

Since many patients are treated outside major academic medical centers and urban hospitals, the RRH hospitals located in smaller communities and remote medically underserved areas hold promise as major sources of biospecimens representative of rural areas, which would be acquired at a relatively lower cost and used for research at the national level. A pilot study initiated in a Swiss regional hospital showed that successful biobanking for research can take place in relatively small regional hospitals without an integrated institute of pathology [20]. Initial cost provided for setup was $35,662 and yearly running cost $1,250. This study also confirmed that in a properly planned setting, acquiring fresh tissue for banking prior to submission for clinical pathological analyses will not compromise patient standard care [20]. Building these kinds of infrastructure in communities will help such communities develop and sustain robust local infrastructure to enhance and promote research activities, thus increasing community participation in research, clinical trials and in a general sense support their role of biobanking in the reduction of health disparities. These public-private RRH partnerships will provide interfacing between administrators, managers, physicians, pathologists, biobankers and scientists leading to enhanced translational research opportunities through the supply of quality biospecimen to advance genomics and proteomics research, identify, and validate biomarkers in addition to the development of new tests and drugs [27].

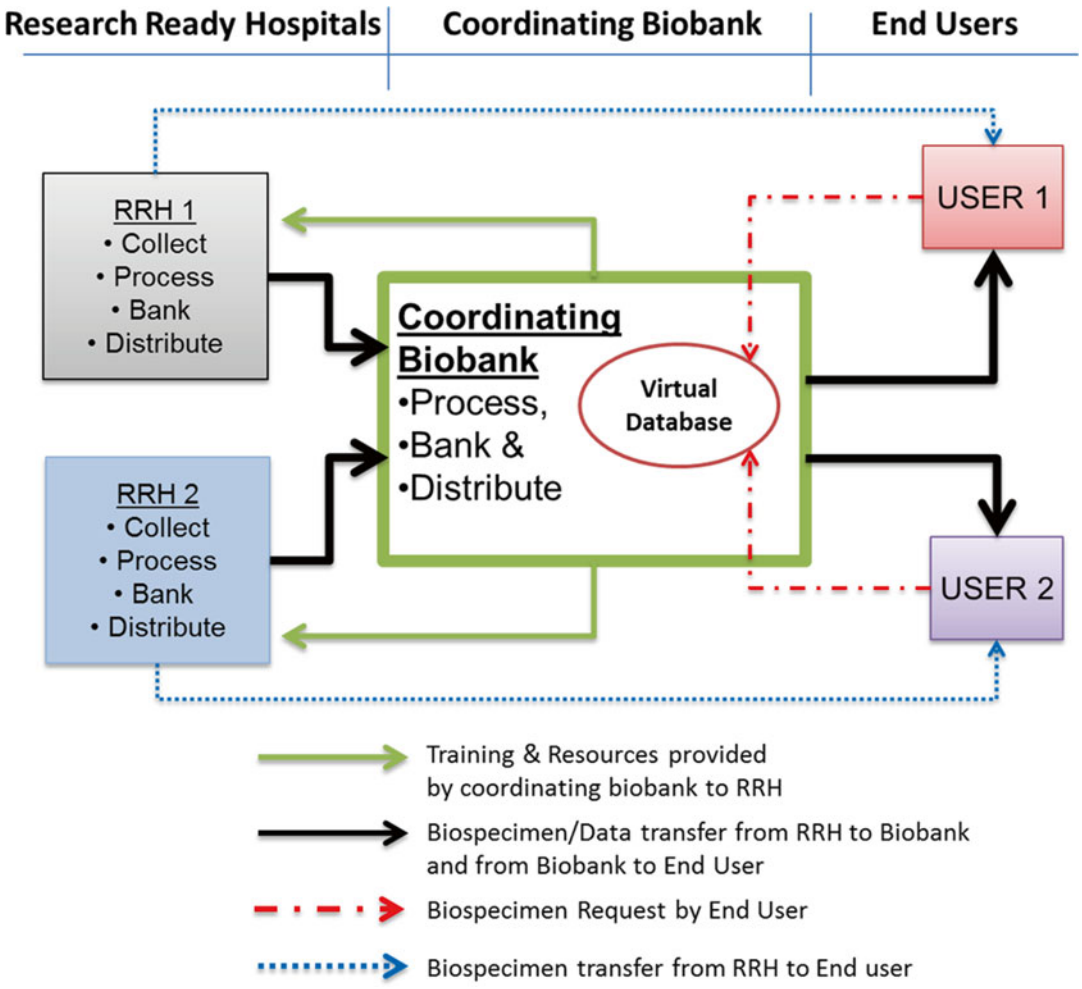

Fig. 2.2 The Research Ready Hospital (RRH) model combines attributes of both the centralized and federated models. The RRH will collect, process, and may temporarily store specimens prior to sending to the Coordinating Biobanking for long term storage, or directly to end users. A virtual database accessible to the Coordinating Biobanks, RRH and end users makes interaction between the three entities seamless. Coordinating Biobanks will provide appropriate training and assistance in the building and operation of the RRHs

2.5.2 Adopting Sustainable Biobanking Practices

Setting up commercial biobanks, national biobanks, biobank networks and Research Ready Hospitals will help increase the number and type of biospecimens available for research. But the cost of operation and the availability of certain specimens will still be scarce due to disease rarity or size restrictions of surgically removed materials. Thus, in addition to developing methods to increase supply and availability in general, biobanks will have to come up with innovative methods to reduce cost of storage and distribution as well as to leverage and extend the impact of already collected rare and precious biospecimens.

All biospecimens are important because they represent a point in time that can never be replicated. To extend the life and impact of each biospecimens it has to be used judiciously, and in a controlled and sustainable manner. One approach to sustainable biospecimen banking is to develop a biospecimen grading system that objectively

Table 2.2 A conceptual grading system that defines value and can be used to control biospecimen distribution by biobanks

Grade	Description	Value	Note
1	No clinical annotation. Not scarce. Easily collected. No limitations in size and number.	Low	Freely distribute.
2	No clinical annotation. Not scarce. Not easily collected. No limitations in size and number.	Low	Freely distribute.
3	Poor clinical annotation. Not scarce. Easily collected. No limitations in size and number.	Low	Freely distribute.
4	Very poor clinical annotation. Relatively scarce. Not easily donated. Some size and number limitations.	Medium	Distribute based on Project importance.
5	Poor clinical annotation. Relatively scarce. Easily collected. No limitations in size and number.	Medium	Distribute based on Project importance.
6	Poor clinical annotation. Relatively scarce. Not easily collected. No limitations in size and number.	Medium	Distribute based on Project importance.
7	Poor clinical annotation. Relatively scarce. Not easily collected. Some limitations in size and number.	Medium	Distribute based on Project importance.
8	Good clinical annotation. Not scarce. Easily collected. No limitations in size and number.	High	Distribute with caution.
9	Very good clinical annotation. Not scarce. Not easily collected. No limitations in size and number.	Very high	Distribute with caution.
10	Extensive clinical annotation. Very rare specimen, difficult to find and collect. Very limited in size and number.	Extremely high	Distribute with highest scrutiny

classifies biospecimens based on some metrics that conveys availability, quality and value. A conceptual "10 point" grading system that utilizes the level of clinical annotation, scarcity/rarity, ease of collection, and availability could be adopted by all biobanks to assign value to biospecimens (Table 2.2). With this grading system, biospecimens could be classified to indicate that; (1) they can be distributed freely due to high abundance in the biobank, (2) distributed based on the scientific merit of the project or (3) distributed with caution due to rarity and difficulty of getting new specimens. Rare and difficult to acquire biospecimens should only be utilized for very high profile projects with great scientific merit and potential to significantly advance knowledge and impact healthcare. Thus a specimen graded as "10" will refer to an "extremely high value" specimen with extensive clinical annotation, difficult to find and collect, and limited in size and number", whereas a specimen graded as "1" will refer to an "extremely low value" specimen with no clinical annotation, not scarce, easily collected and no limitations in size and number.

The use of such a simplified and objective grading system will help with biospecimen management, and ultimately the harmonization of best practices in the area of estimating the value of a biospecimen based on the ease of collection, rarity of the specimen and potential impact when used for research. It will be an objective way to compare the value and cost of different biospecimens, manage the distribution of rare specimens and communicate with peers. For example, since not every research requires and should use whole tissue sections or biospecimens with extensive clinical annotation, it will be necessary to limit and control the distribution of rare and precious specimens (e.g. Nos. 8–10) so that they will be available for high impact studies. Basic, feasibility and proof-of-concept studies where the primary

objective of the study is to establish for example analytical sensitivity of an instrument should not use rare and previous biospecimens, or even a commonly available biospecimen with the level of clinical annotation that is required for studies concerned with disease progression, outcome and clinical end stage. Low value specimens e.g. Nos. 1–3, will be expected to be less expensive to acquire and distribute. Biospecimens in groups Nos. 1–3 though perceived as low in value, probably because of for example, limited or incomplete clinical annotation, are still very useful to researchers because they were collected, handled and stored using best practices and hence still have other attractive quality attributes associated with biobank supplied specimens.

Considering that the size of certain specimens e.g. breast biopsies are getting smaller due to earlier detection with mammography, biospecimen banks will have to adopt different strategies to extend the usage of the limited number or size of specimens available. Not all specimens requested by scientists will be studied at the DNA, RNA and protein levels. Thus, rather than send a tissue section to a lab that performs studies only at the DNA level, biobanks could utilize a protocol that allows the isolation of DNA, RNA and proteins from the tissue, send an aliquot of the DNA to the requesting lab and retain the remaining DNA, as well as all of the RNA and protein for future studies.

It would also be necessary to use established or new methods to distribute biospecimens or the DNA, RNA and proteins derived from them. It has been demonstrated that "touch preparations" on microscope glass slides and FTA paper can provide sufficient and good quality DNA suitable for PCR and probably other downstream applications [25]. That means biobanks can support a larger number of research studies that require DNA by sending touch preparations on paper or glass slides rather than sending the entire specimen. The cost of distributing tissue imprints will also be lower since there will be no requirement to send samples with dry ice.

Many biobanks still utilize frozen storage as the main method for preserving biosamples like DNA and RNA. Cold and frozen storage is expensive, requires a lot of space and time, and may not be necessary all the time. Accumulating evidence indicate that not all biospecimens need to be stored by freezing. In fact DNA stored in the dry state at ambient temperature, and distributed in the dry state can be used for many downstream applications. Recent studies also indicate that RNA and proteins can be stored and distributed in the dry state and at ambient temperature. There are now many companies including GenTegra LLC that manufacture products for dry state and ambient temperature storage of DNA and RNA.

2.6 Conclusion

A well planned biobank that delivers quality biospecimens is a critical resource in advancing biomedical science. The requirement for readily available and accessible, good quality, human tissue to feed today's high throughput technologies continues to be on the rise. The collection and handling of these biospecimens is of strategic importance as the accuracy of the data generated from them depends on the quality of the biospecimen. While the issue of biobanking standards can take up entire publications, the aim of this chapter is not to delve into the complete details of standardization and quality but to look into ways of increasing the supply of good quality and different types of biospecimen at a cost that can be afforded by most researchers. The availability of good and well annotated biospecimens for research will speed up the discovery of new biomarkers, and targets for innovative therapies [28, 29]. The only way to increase the number and types of biospecimens is to increase the number of "tissue sources". The establishment of RRH that can supply biospecimens on demand could increase supply without significantly increasing operating expense of biobanks. The current idea represents a topic that should generate conversation in the industry. This conversation will then lead to the development of the finer details required for the establishment of RRH. The legal, regulatory and economics of biobanking is still an area to be fully developed in the industry, and

the proposed RRH will need to be established with appropriate knowledge of the associated direct and indirect cost implications of establishing and maintaining physical and virtual biobank networks which can be activated on demand.

Sustainability issues will need to be addressed and how cost recovery practices and funding will be achieved by all stakeholders. The success of this endeavor will lie greatly on the success of the established public-private partnerships and the aims and goals of the business relationship between the coordinating biobank and the RRH. The partners will have to recognize the fact that they are in the business of providing high quality biospecimen to drive research that leads to better health for future patients. The idea of embedding biobanking in the healthcare system has been suggested [30], an indication that biobanking efforts need to be expanded into many more hospitals. Organ donation organizations are already successfully operating within numerous hospitals in the United States, to recover organs (lung, kidney, heart etc) and tissue (cornea, bone/tendon, heart valves etc) for transplantation. Since CAP is already the accreditation agency for hospitals and biobanks in the United States, very little resistance, if any, is therefore anticipated when creating RRH that will leverage an existing hospital's infrastructure and system to more systematically procure specimens to support biomedical research. By adopting new methods and technology for biobanking and distribution of biospecimens, the biobanks of the future will be able to reduce space requirements, energy consumption and the overall cost of operation. This will allow them to extend the life of biospecimens and support many more researchers in a sustainable manner.

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Sustainability of Biobanks in the Future

3

Yvonne G. De Souza

Abstract

Human biorepositories are essential in providing high quality specimens that are well characterized. Biospecimens are used in basic, clinical, and translational research. However, as regulatory requirements and scientific demands increase the complexity of the daily operations of a biorepository, the cost of maintaining a biobank will increase. How can biobanks today maintain sustainability during the current economic climate and changing landscape of operating a biorepository? This is a brief review of how different biobanks have approached sustainability.

Keywords

Biobank • Biorepository • Cost recovery • Fee-for-service • Sustainability • Workflow

3.1 Introduction

The focus of this paper will be on the future sustainability of human biobanks/biorepositories. Human biobanks have evolved over the past decades. The majority of biobanks started as small academic biorepositories that were developed for specific or unique research projects. Over time biobanks evolved to larger institutional, government supported biorepositories, commercial biorepositories (for profit), population based biobanks, and virtual biobanks. Their basic mission is to collect, process, store, and disseminate human specimens and data that are used for basic science and biomedical studies. These specimens play an important role in the development of new therapeutics, pharmaceuticals, diagnostics, population genomics, etc.

The field of biorepository and biospecimen science keeps evolving due to changing needs of researchers, regulatory requirements, ethical and legal issues, and the rapidly changing face of science [1]. The disciplines of proteomics,

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genomics, and personalized medicine all demand high quality specimens associated with well characterized data. Today there is discussion among funding agencies that biorepositories should obtain accreditation [2, 3] in order to demonstrate consistency in quality assurance and quality control programs.

However, as regulatory requirements and scientific demands increase the complexity of daily operations of a biorepository, the cost of processing and maintenance will increase. How will biobanks of the future sustain themselves? The economic down turn of 2008 has affected nonprofit and for profit biorepositories. Today large pharmaceutical companies have reduced staffing as well as research and development programs, and academic biobanks are experiencing reduced funding from institutional and government agencies. As market forces change the business structure, the character, and morphology of a biobank will have to change, to meet the ever increasing need for biobank innovation and services.

3.2 Economics of a Biorepository

Biorepositories are costly in regards to staffing, equipment, service contracts, consumables, and expertise [4–6]. For many biorepositories a preeminent expense is that of maintaining a collection of specimens (long term storage) that are under-utilized. In order to maintain sustainability, biobanks must run as business units as well as scientific laboratories [7]. Some academic biobanks outsource their storage of collections. To maintain sustainability some biobanks leverage the financial potential of their specimens and data. However this may lead to ethical and legal issues in regards to HIPAA, consent forms, and the public trust in biorepositories [8].

Some recently published works have described various operational models that may provide insight as how to sustain a biorepository. Vaught et al. [4] reviewed 16 of the largest international biobanks and networks in their management of processing and storing biospecimens while recovering their operating costs. The biobanks reviewed have agreed that the specimens they store cannot be used for commercial purposes. The majority of biobanks implemented a cost recovery system by charging investigators access to specimens and data. However, all of the biobanks reviewed did not fully recover their costs. They relied on governmental and charitable support. The approaches to cost recovery varied among the many biobanks. Some defrayed the cost of a portion of the price of biobanking in order to make their services affordable to the investigator. Other biorepositories had different cost recovery rates for non-profit versus private companies. Additional sample processing services were offered by some biobanks in which the full cost was paid by the requestor.

3.3 Academic Biorepositories

For an academic biobank, a fee for service [7] model is one approach to recover a biobank's expenses. Federal funding is shrinking and charitable donations are few and far between. At the University of California, San Francisco (UCSF), core facilities are encouraged to develop a recharge methodology in order to recover their costs. A recharge will recover nonsubsidized direct costs for a core's services. A recharge proposal is submitted to UCSF Budget Office for review and approval. The UCSF AIDS Specimen Bank (ASB) has developed a recharge methodology to recover its costs associated with staffing, processing, consumables, equipment depreciation, service contracts, software and hardware maintenance, data management, storage, and dissemination. In the development of this recharge the workflow of ASB had to be taken into consideration in determining a fee-for-service schedule.

Figure 3.1 depicts the work flow of receiving, processing, and storing specimens. Figure 3.2 depicts the dissemination process in which specimens are selected, removed from storage, and shipped to their final destination. Each step in the process has a related cost.

At the Washington University Medical Center, St. Louis, Missouri's Tissue Procurement Core a fee- for- service business model [7] was developed

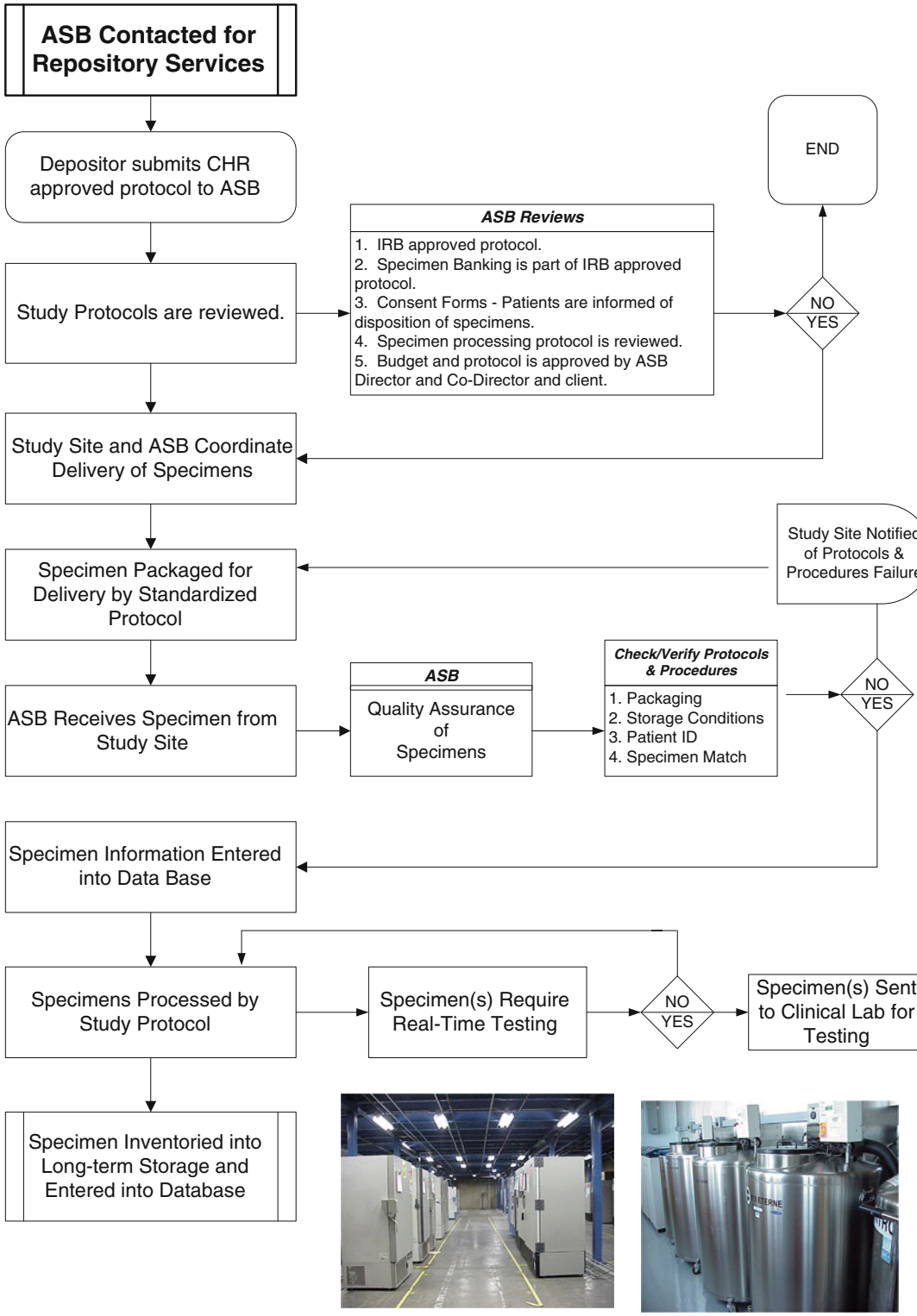

Fig. 3.1 UCSF AIDS specimen bank – specimen accessioning, processing and storage workflow

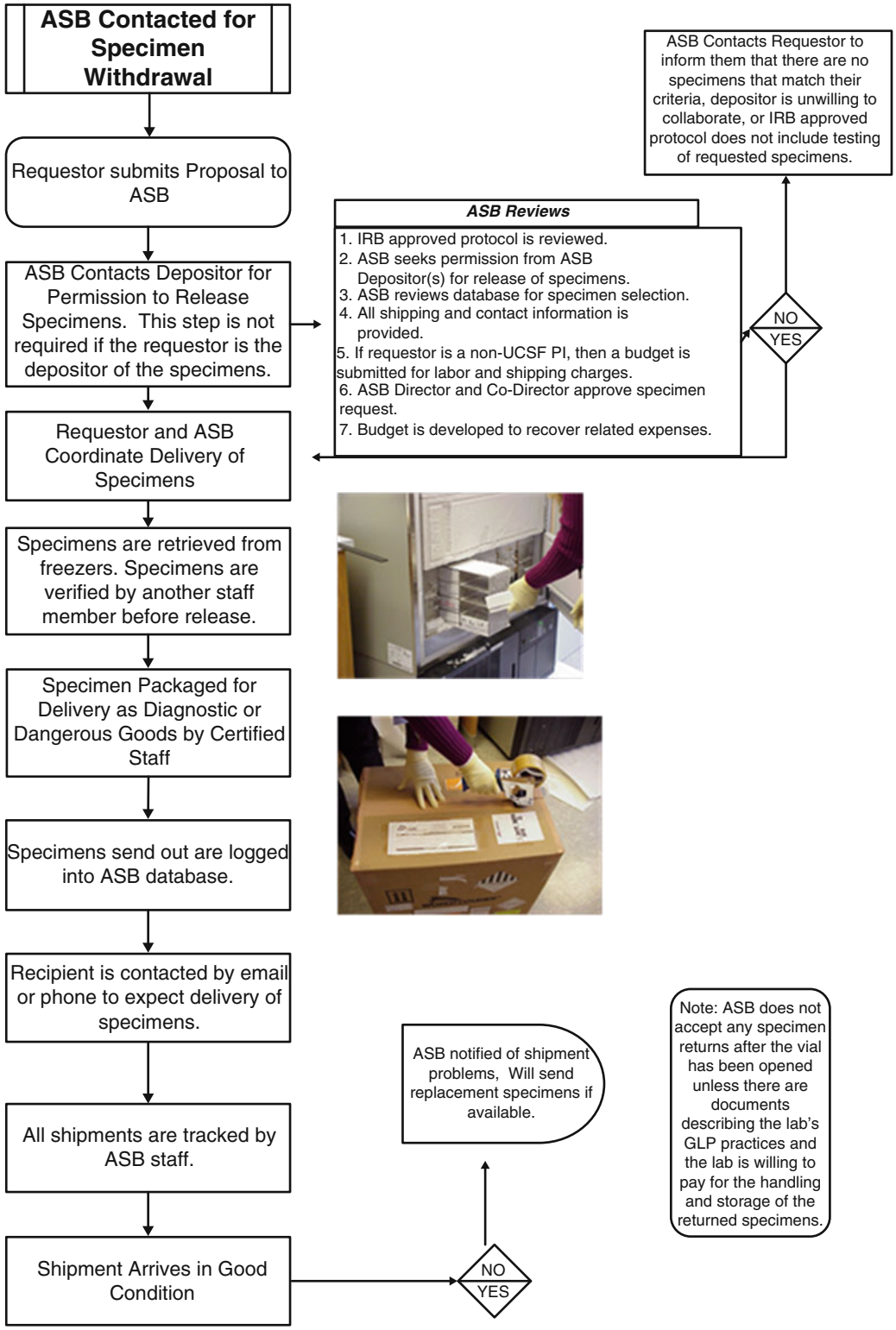

Fig. 3.2 UCSF AIDS specimen bank – specimen withdrawal/request workflow

in order to recover operational costs while still offering competitive value to its users. They do not charge researchers for the use of biospecimens, but rather the services associated with the specimens. They developed a financial model taking into consideration labor, consumables, pathology review, storage, and infrastructure.

McQueen et al. [9] describes the challenges that arise when managing and sustaining a large biobank and their Clinical Research Trials Laboratory (CRTL) at the Hamilton General Hospital in Hamilton, Ontario, Canada. Their biggest challenge was obtaining space for freezers and laboratory space. Their bank grew from 500 ft^2 in the 1990s to about 12,000 ft^2 in 2013. The Hamilton Health Sciences provided the space, and there is support from industry due to the high quality of the clinical studies being developed by the CRTL. The Population Health Research Institute (PHRI) also provided support as well as grants. This paper describes how collaboration, the implementation of best practices as published by the International Society of Biological and Environmental Repositories (ISBER) [10] and the National Cancer Institute (NCI) [11] help them to achieve accreditation of their biobank and CRTL by the International Standards Organization (ISO). By achieving accreditation and producing high quality specimens, this biobank continues to sustain itself.

Development of a centralized and well-coordinated biorepository within an academic institution may be an approach to reduce costs and improve the quality of specimens and its associated data. A centralized biobank may help to promote collaboration among investigators [12]. A common informatics system will help to direct or manage the collection, processing, and dissemination of biospecimens within an institution.

The centralization of an academic biorepository does not necessarily mean that one biorepository will serve the needs of an academic institution. The process of centralization could be that an institution would invest in a common informatics system that will link biobanks and researchers. This would improve the coordination between researchers and access to biospecimens. Standard operating procedures would be shared and best practices would be developed for quality control and quality assurance. This could help to improve the sustainability of an institution's biorepositories by insuring that the specimens processed are of high quality and are well characterized.

3.4 Other Economic Models

Several publications from the NCI [13–15] describe key considerations in the development of a cost recovery model for a biorepository. Factors such as size of the biobank, inventory turnover, market price, and other potential revenue sources are discussed.

The Infectious Diseases Biobank (IDB) at King's College London [16] developed an interesting economic model in order to sustain their extensive tissue collections. In addition to their core funding the IDB developed three strategies to increase their funding. The first was to charge investigators for samples and associated labor, or an investigator could donate specimens to the IDB. The next step was to identify 'emerging markets' outside the original scope of the IDB. Their third step was the most successful, was the offering of contract services.

Watson et al. [17] published a paper in which they proposed that sustainability of a biobank must take into consideration a framework which includes financial, operational, and social. Financial would include developing a business model defining user fees based on operational costs, identify stakeholder needs, measure value and monitor its impact on the biorepository. Operational decisions would involve reviewing and improving the biobanking process. This includes specimen collection and processing, data annotation, and assessing if a biobank needs to offer more products and services. The social aspect refers to the impact of a biobank to the community, its participants, patients that donate specimens, and finally the funding agencies.

The British Columbia BioLibrary [18] (BC) was created to connect specimen donors, biobanks, and researchers. It is not a biorepository

but more of a conduit that has enhanced the value and accessibility of high quality biospecimens to investigators and has gained the public's trust. This has contributed to the sustainability of biobanking in British Columbia.

The University of British Columbia Office of Biobank Education and Research [19] developed a biospecimen user fee calculator that could help biobanks develop a more accurate and transparent costing tool. They enrolled several members of the Canadian Tumour Registry Network (CTRNet) to test the tool. The authors commented that many biobanks keep their prices low in order to increase business and investigators that request services from a biorepository may have not planned to pay for these services in their grant proposals or had inadequately budgeted for these services. These inadequate planning issues will not financially sustain a biorepository. The authors this tool available on line at www.biobanking.org. This tool is designed to give a biobank the ability to develop a realistic fee for their services. There will be additional releases of this tool in the future.

3.5 Conclusion

During these challenging economic times it is essential that biobanks develop an efficient cost recovery mechanism in order to remain sustainable. The NCI's Biorepositories and Biospecimen Research Branch has developed a financial sustainability survey to collect data on direct and indirect costs associated with biobanks, technology challenges associated with the operations of the biobank, demographic data of biobanks, and techniques that biobanks have used to successfully maintain financial sustainability.

In order to remain sustainable a biobank must communicate with their customers and stakeholders to gain support for their methodology of cost recovery. In addition, biobanks must develop viable business models and marketing strategies. These methods must be reviewed annually to adjust to changes in client needs. There is no one perfect solution in maintaining sustainability. It is imperative biobank managers must understand the complexities of science and business in operating a biorepository.

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Biobanking: The Future of Cell Preservation Strategies

4

John M. Baust, William L. Corwin, Robert VanBuskirk, and John G. Baust

Abstract

With established techniques cryopreservation is often viewed as an "old school" discipline yet modern cryopreservation is undergoing another scientific and technology development growth phase. In this regard, today's cryopreservation processes and cryopreserved products are found at the forefront of research in the areas of discovery science, stem cell research, diagnostic development and personalized medicine. As the utilization of cryopreserved cells continues to increase, the demands placed on the biobanking industry are increasing and evolving at an accelerated rate. No longer are samples providing for high immediate post-thaw viability adequate. Researchers are now requiring samples where not only is there high cell recovery but that the product recovered is physiologically and biochemically identical to its pre-freeze state at the genominic, proteomic, structural, functional and reproductive levels. Given this, biobanks are now facing the challenge of adapting strategies and protocols to address

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these needs moving forward. Recent studies have shown that the control and direction of the molecular response of cells to cryopreservation significantly impacts final outcome. This chapter provides an overview of the molecular stress responses of cells to cryopreservation, the impact of the apoptotic and necrotic cell death continuum and how studies focused on the targeted modulation of common and/or cell specific responses to freezing temperatures provide a path to improving sample quality and utility. This line of investigation has provided a new direction and molecular-based foundation guiding new research, technology development and procedures. As the use of and the knowledge base surrounding cryopreservation continues to expand, this path will continue to provide for improvements in overall efficacy and outcome.

Keywords

Cryopreservation • Apoptosis • Molecular control • Biopreservation • Thawing • Cell storage • Cryopreservation induced cell death • Freeze injury • Improved survival • Necroptosis

Abbreviations

CCM	Complex cryopreservation media
CIDOCD	Cryopreservation
CPA	Cryoprotective agent
DMSO	Dimethyl sulfoxide
DOCD	Delayed onset cell death
ISBER	International Society of Biological and Environmental Repositories
LN_2	Liquid nitrogen
NCI	National Cancer
NIH	National Institutes of Health
TAC	Target apoptotic control
Tg	Glass transition temperature
UPR	Unfolded protein response

4.1 Introduction

Whether a biobank exists as an asset of an individual research laboratory or as an "industry/not-for-profit/government" mega-bank archiving an extensive diversity of samples, its purpose is to preserve biological specimens with the expectation of future recovery to support knowledge development relevant to a purpose such as disease control. In view of the diversity of sample types and methodologies available for banking, the concept of "fit for purpose" often serves as the strategic guide for the selection of sample processing methodologies. Implicit in this approach, but often unstated, is the probability that the samples may best serve other (unanticipated) purposes in the decades ahead. Today, biobanking preservation strategies should portend accurate predictions of future needs. In effect, tomorrow's successes will be dependent on the application of current mid-twenty century methodologies of preservation, which, unfortunately, often yield samples of limited utility [1–3]. How then do we reconcile the uncertainty, and therefore assure future utility of many millions of cryopreserved mammalian cell collections?

Today's biopreservation is characterized by a diverse scientific foundation integrating the fields of cryobiology, engineering, computer sciences, structural chemistry and cell/molecular biology [1, 3, 4]. Successful biopreservation requires the effective use of or the development of methodologies that support the preservation of cells, tissues and organs with post-storage return to pre-storage functionality [2, 3, 5]. Biopreservation is characterized by rapid growth as advances in cell therapy, stem-cell research, personalized medicine, cell banking, cancer research, etc. drive the need for optimized storage protocols. However, the field still experiences significant

problems with the current techniques including: sub-optimal survival, loss of cell function post-storage, addition of animal components in storage solutions, and activation of cellular stress pathways which can lead to alterations in gene expression, protein composition, micro RNA's, etc. [4, 6].

The first successes in cryopreservation can be traced to the Polge et al. [7] serendipitous discovery that avian spermatozoa could be successfully preserved in 20 % glycerol when pelleted on to a block of dry ice and a subsequent report on human erythrocytes [8]. In the decades that followed scores of empirical studies investigated process manipulation of two atypical cell types (the human RBC and spermatozoa) from diverse species. These cell types served as the models of choice due clinical/agricultural need, ease of experimental manipulation and availability. In 1959 Lovelock and Bishop [9] reported on the first use of dimethyl sulfoxide (DMSO) as a cryoprotective agent. Successful cryopreservation of spermatozoa is assessed not by normal physiologic function but by artificial insemination outcome which effectively requires only that acrosomal degradative enzymatic activity and DNA integrity be maintained. RBC survival was determined by percent hemolysis based on hemoglobin leakage immediately post-thaw. Long term viability of thawed RBC remains uncertain. Fortunately, and some might counter unfortunately, the application of these "… *penetrating cryoprotectants … enabled empirical cryoprotection to leap frog basic research*" [2].

In the decades that followed numerous hypotheses were proposed to account for freeze injury. These included: Lovelock's salt concentration, Meryman's "minimal cell volume" [10], Mazur's "two-factor hypothesis" [11, 12] and Steponkus' "membrane deletion concept" [13]. Mazur's two-factor hypothesis remains widely accepted amongst research cryobiologists as one of the "factors," rapid cooling rates fail to permit adequate water efflux from cells resulting in the formation of lethal intracellular ice [12]. The second factor focused on the toxic "solution effect" which predicts that if cells experience long exposures to concentrated solutes such as cryoprotectants, solute toxicity will be evident.

Accordingly, an optimal cooling rate would be required to minimize the destructive consequence of cooling at either too rapid (= intracellular ice) or too slow (= cryoprotectant toxicity) a rate. What followed were thousands of studies on the biophysical aspects of changes in cell volume and calculated water efflux rates with various cryoprotectants, cell types, cooling rates and strategies for cryoprotectant addition and removal. The goal since the 1960s has been to manage cellular water to prevent intracellular ice formation, and as a consequence of solute freeze concentration, to lower preservation temperatures below the nominal glass transition (Tg) range for pure water (Tg = ~ −135 °C) [14]. Below Tg "liquids" (also referred to as amorphous or acrystalline solids) are of high viscosity (10^{12} Pas) and reactions are determined by diffusion kinetics (WLF kinetics) rather than energetically driven Arrhenius kinetics. Since, for example, below Tg the rate of diffusion of a proton (hydrogen ion) has been estimated to take over 200 years to move one molecular diameter, chemical reactions are improbable. To terminate low temperature storage, cells are rapidly thawed to both minimize time above Tg thereby preventing energetically driven reactions, devitrification (ice formation at temperatures just above Tg) and recrystallization (disparities in ice crystal surface energy that causes changes in ice crystal size). Ice-free preservation, a form of vitrification, is designed to eliminate extracellular ice by exposing cells to very high solute concentrations (up to 8 M) in a time specific manner thereby minimizing the "solution effects" above Tg while relying on increased viscosity to suppress ice crystal growth [15, 16].

While the above strategies generally yield survival as measured immediately post-thaw [6], many cells in most samples continue to die over the following 24–48 h (Delayed On-set Cell Death) [17–20]. Surviving cells may lose key functional characteristics that may not be recovered until following generations [21, 22]. As a result studies focused nearly exclusively on structural parameters effectively treat cells as passive participant in the preservation process, as passive osmometers, thereby ignoring the critical

biological responses to the severe oxidative stress of cryopreservation. In effect, every processing step beginning with cell harvest through chilling, cryoprotectant exposure, freezing to Tg and thawing, *the cold chain*, occurs within the "hypothermic continuum" characterized by multiple cellular survival/death responses. Today, and in the future, it will be the management of these biological cascades that will determine not just whether or not a cell survives but whether or not it retains normophysiologic function. Several distinct mechanisms of cell death are now recognized following the application of an "optimized" cryopreservation protocol: physical cell rupture, apoptosis and necrosis [17, 20, 23–25]. Other possible forms of cell death such as autophagy, anoikis and more recently necroptosis may also be associated with cryopreservation but necessitate further study.

4.2 Hypothermic Storage

Hypothermic storage is primarily a metabolic suppression strategy for the maintenance of biological material. While the protective effects of cold have been documented for centuries, our understanding of the biological consequences of cold exposure is relatively recent. The "modern era" of low temperature cell preservation began with Carrel's investigations on the perfusion of organs prior to transplantation which related the characteristics of a perfusion medium [26–28].

As cold preservation entered the modern era, both hypothermic and cryopreservation techniques were developed to increase storage intervals by limiting the negative effects (i.e. ischemia, hypoxia, etc.) associated with cell and tissue harvest and isolation [1, 3, 28]. The central principle underlying the use of cold as a preservation tool is grounded in the reversible depression of cellular functions. Cryopreservation relies on ultralow temperatures to bring a cell's metabolism to a halt in support of an indefinite storage period. However, the current state of cryopreservation is only effective for single cell suspensions and a few simple tissues [1]. The cryopreservation of complex tissues or whole organs results are limited by "cryoinjury," manifest by cell death and the loss of higher order functions [4, 29]. For complex biologics hypothermic storage remains the most effect strategy for the preservation [1, 2, 28, 30].

The process of whole organ preservation requires an initial step of cold perfusion upon harvest. The hypothermic preservation solution supports transport prior to implantation at which time a warm reperfusion process flushes the hypothermic solution from the organ and returns it to normothermic temperature prior to implantation. While this method has proven to be far superior to warm perfusion and storage, it supports very limited preservation times (hours to days) [28]. Progress in preservation solution design was dependent on advances in the recognition of cellular responses to stress and a growing knowledge of tolerable limits that challenge normophysiological processes. This focus led to the development of the first intracellular-like cold perfusion solution, University of Wisconsin solution (ViaSpan®) which remains the "gold standard" of preservation solutions for many organ systems [3, 28, 30] since the late 1980s. While a physiological approach to preservation solution design continues [3, 27], there remains a significant limitations in complex tissue/organ preservation [3, 28, 30]. More recently, advancements in our understanding of the molecular response of cells to cold are supporting targeted approaches (cell and tissue specific) to extended preservation intervals and higher quality "product" [1, 3, 25].

4.3 Hypothermic Continuum

The medical literature generally defines hypothermia as mild (32–35 °C), moderate (27–32 °C), deep or profound (10–27 °C) and ultraprofound (0–10 °C) [2, 28]. We suggest that these divisions at above freezing temperatures along with differences in the aims of hypothermia and cryopreservation, have led to "disciplinary isolation" by those focused on these distinct preserva-

tion strategies [3]. As noted, the successes of hypothermic preservation have made significant gains through the understanding of fundamental cellular processes. Cryopreservation, on the other hand, has historically focused on the physical aspects of freezing leaving a disconnect between these related fields of study.

In cryopreservation research a distinction between pre- and post-freeze chilling exists. This distinction provides an artificial boundary at the temperature at which extracellular ice is manifest (nominally −2 °C). For a cryopreservation protocol to be "successful," intracellular freezing must be avoided whether by freeze concentration of the protective solute or the addition of initial high concentrations of solute. Both strategies result in intracellular vitrification. As a prelude to cryopreservation, the biologic transitions from its normothermic state (typically 37 °C) to hypothermic temperatures (~0 to 10 °C). This initial cooling can provide short-term benefit such as decreased metabolism, reduced oxygen consumption, and reduced nutrient demand thereby increasing overall survival. Cold exposure does, however, initiate numerous negative effects correlated the change in the energy state. Lowered temperature yields a decrease in the kinetic energy necessary to support normal physiological reactions resulting in a depletion of ATP [25, 28, 29, 31]. Early targets of hypothermic damage include cell membrane structure changes from liquid-crystalline to solid gel-like state, and functionally, as membrane mediated transport fails ionic imbalances become pronounced [2, 28, 30]. Increase levels in cellular calcium and sodium, losses in potassium and intracellular acidosis (pH approaching 4) occurs [28]. Numerous other disruptive events occur simultaneously within the cell including the leakage of hydrolases, generation of free radicals, disruption of cytoskeletal elements and mitochondrial-linked events leading to the activation of apoptotic machinery [4, 32–34]. With prolonged chilling, many molecular-based responses will activate or be suppressed which must be recognized and possibly be altered through molecular-based strategies to assure post-thaw survival [3, 4, 28, 35, 36].

4.4 Cryopreservation Process

Cryopreservation is a technique for maintaining biologics at cryogenic temperatures (at or below −80 °C, for prolonged periods of time). Cryopreservation processes begins with the exposure (~10 to 30 min) of cells to a cold cryopreservation solution. The process proceeds with further cooling to extend the hypothermic continuum to the storage temperature. Equilibrium is reached as the system reaches a glassy or vitrified state. As discussed, cells experience profound stress during the cooling interval of the cryopreservation process (up to 2 h) before reaching Tg where it is assumed that there are no further deleterious effects. It is often unappreciated that in order for a cell to be successfully cryopreserved the cell itself must avoid freezing, therefore remaining in a state of deepening hypothermia until Tg is reached. In essence, for a cell to be successfully cryopreserved, it must remain in a ultra-cold liquidous state until transitioning to a glassy state. Generally speaking, if ice forms within a cell during any part of the process, survival will be compromised.

Cryoprotective agents (CPAs) function, in part, to lower the probability of intracellular ice formation. Since glycerol was described as an effective CPA for both avian spermatozoa and human erythrocytes, numerous other compounds have been identified as CPAs. Today's, CPAs include a variety of penetrating (membrane permeable) and non-penetrating compounds contained in an appropriate cell culture media [6, 19, 37] with or without serum. An optimal cooling rate, nominally 1 °C per minute, is commonly applied for mammalian cell [12, 29, 31]. This allows for cellular dehydration and reduces the probability of intracellular ice formation which otherwise would cause cells to rupture upon thawing [12]. While generally accepted, studies by Acker et al. [38] have shown that some cells can tolerate a limited amount of intracellular ice during the process. While avoidance of intracellular ice is critical, if cooling rates are too slow, prolonged exposure to high solute concentrations can result in toxic effects (i.e. "solution effects") [3, 12].

Advancements in cryopreservation over the last several decades have helped to "optimize the process" yet have yielded varying degrees of success [2, 3, 5, 19, 29]. The most commonly practiced process is as follows: (a) cells are incubated in a culture media containing a cryoprotective agent such as DMSO (dimethyl sulfoxide), the most commonly employed CPA. To this end, over the last decade there has been a paradigm shift in solution design to include "intracellular-like" solutions as the CPA carrier media as a substitute for traditional culture media [18, 22, 39, 40]. As discussed later in this chapter, studies have shown that this shift in carrier solution design yield a significant increase in post-thaw cell survival and function [18, 21, 39, 40]. Following (b) a 10–30 min incubation at 4 °C, the cells are cooled (typically) at a uniform rate of 1 °C/min. A uniform cooling rate may be produced in an active or passive manner. Programmable controlled rate coolers provide active cooling. These devices monitor sample temperature and vary cryogen injection to provide a pre-determined cooling rate. Passive cooling methods utilize containers in which samples are surrounded by, but isolated from, alcohol. The container (c) is placed into a −80 °C freezer to achieve an approximate cooling profile of −1 °C/min. Ice nucleation (seeding) is often performed between −2 and −6 °C to prevent damage associated "flash" freezing due to sample supercooling. Seeding in active cooling devices is initiated through a programmed, thermal shock to the samples or through physical agitation of samples passive devices. Cooling (d) continues at a controlled rate to a predetermined temperature (i.e. −40 to −80 °C). Samples (e) are then transferred to ultralow temperature storage (i.e. liquid nitrogen immersion, liquid nitrogen vapor, or mechanical storage of < −135 °C). These ultralow temperatures fall below the reported glass transition temperature (Tg) of pure water [14, 41, 42] which arrests all molecular interactions (i.e. metabolism) and is thought to prevent a free radical generation [3]. In the glassy or vitrified state the viscosity of the solution is high causing the translational motion of molecules to cease. After storage, the cryopreserved sample is rapidly thawed to limit further exposure to negative effects associated with chilled liquid state. Sample thawing while agitated in a 37–40 °C water bath progresses until the last ice crystal is observed. Dilution of the sample is then accomplished by the addition of fresh culture media. New innovations in thawing rely on programmable thaw devices that provide repeatable, uniform and documentable sample thawing are discussed below.

4.5 Vitrification Strategies

Sample vitrification may be attained with an alternate technique. With this alternate technique a step-wise addition of high molar concentrations of cryoprotectant during the cooling process achieves an "ice-free" state [15, 16]. While similar in aim, vitrification procedures are different from that of controlled rate cooling. It has been shown that both extracellular and intracellular ice formed during cooling are damaging. The avoidance of ice formation makes vitrification a potentially viable option for the preservation of more complex tissues [15, 16]. A detailed discussion of vitrification is beyond the scope of this chapter. For additional discussion on vitrification procedures we refer the readers to articles [43–47].

4.6 Sample Thawing

One aspect of the cryopreservation process which received little attention is that of sample thawing. It is well established that rapid thawing of samples provides for improved cell viability post thaw compared to slow rates [11, 48–51]. Rapid warming rates allow for thawing of samples while minimizing recrystallization of ice and cellular exposure time to high osmolality and CPA concentrations. Rapid thawing is most often achieved via removal of samples from storage followed by immediate placement into a warm (37 °C) water bath. The time between removal from cryogenic temperatures to placement into the warm bath ("air time") is critical and should be kept as short as possible (few seconds). Prolonged time (>30 s, nominally) at tempera-

tures above −80 °C results in slow sample warming which can compromise cell viability and function. To this end, protocols often call for the transfer and transport of frozen samples in dry ice (−79 °C) or in LN_2 (LN_2 baths or dry shippers) to maintain the ultra-cold temperature of the sample until immediately before thawing. As described, the most common thaw procedure is to place samples into a warm (37 °C) bath. Gentle mixing or agitation is recommended to reduce the formation of steep thermal gradients within a sample throughout the thawing process. This prevents the formation of a microenvironment within a given sample where a portion of the sample is exposed to elevated temperatures (approx. >10 °C) where CPA's can be toxic. Samples are held in the warm bath until the last bit of visible ice has dissipated at which point samples should be removed and placed into a cool rack or on ice until dilution in culture media and plating or use. While thawing using a warm water bath has been practiced for over 50 years, this process is being reexamined as the "art of thawing" is not compatible with today's regulated and documentation intensive research and clinical environments. To address this need, a number of devices are being developed to provide for rapid, controllable, repeatable and documented sample thawing. These devices fall into the classification of "dry thawers" wherein samples are warmed in a dry heated chamber. While concerns over the reduced heat transfer efficiency of dry thawers compared to wet water baths have been expressed, reported thaw rates are comparable between the approaches. Further, dry thawers offer a number of advantages over water baths including improved processing and reduced risk of contamination and user error among others. To this end, BioCision recently introduced the *ThawSTAR* system designed to rapidly thaw a single cryovial while reportedly providing for a recorded thermal history of the sample [52]. In the blood banking arena, several dry thaw systems are available (*Plasmatherm*, *Sahara III*, *CytoTherm*) which utilize heated metal plates to thaw frozen blood product bag samples. Most recently, the *SmartThaw* system has been introduced as a next generation dry thawing device supporting the thawing of multiple container configurations (vials, bags, ampules, syringes, etc.) [53]. The *SmartThaw* system achieves rapid thawing of sample vials (1–4 vials), 25 ml cell therapy bags, 250 ml blood bags among others via a soft compliant thaw surface interface between which samples are placed. This compliant interface results in a sandwiching of a sample and provides for 360° of uniform warming. The system also provides for gentle agitation of the sample during the thaw interval. Like the *ThawSTAR* cryovial thawing system, the *SmartThaw* device provides a downloadable sample thermal history allowing for documentation of the thaw process. While ultimately these systems provide a similar outcome to that of water bath approaches, these dry thawers provide for more consistent and repeatable sample thawing which is documentable and can be performed in a clean or even sterile manner which is not possible with water bath approaches. The shift to dry thawing systems will enable end users to recover the highest quality sample possible while reducing the risk of sample loss, contamination, user error, thereby eliminating the "art" necessary for sample thawing.

4.7 Post-storage Outcome

Over the past half century, the improvements in cell preservation technologies have been modest with significant challenges remaining to be overcome. Cells post-thaw often appear viable in the hour or two after thawing. However, when examined 24–48 h later, a significant portion (30–70 %) of these cells succumb to delayed-onset cell death (DOCD) [6, 20, 25]. In effect "optimized" cryopreserved processes do protect cellular structure but fail to adequately manage the biological stresses associated with cryopreservation. An inability to manage the oxidative stresses attendant to cryopreservation results in the delayed initiation of complex cell death cascades leading to a loss of viability [4, 20, 25].

Studies have shown that the delayed molecular effects following thawing extend beyond that of cell survival or death, but impact function as well. Overall function of cellular systems following

cryopreservation has been an issue often overlooked due to the immediate challenges presented by working to improve "survival". The literature contains numerous reports citing high post-thaw cell viability and function [4]. Further examination of these studies, however, reveals that in many cases there are significant compromises in function post-thaw in cell systems such as hepatocytes [22, 54, 55], pancreatic islets [56], cardiac cells [57], blood cells [58], and stem cells [59]. Abrahamsen et al. [60] used flow cytometry to assess sample quality levels (apoptosis and necrosis) following cryopreservation as a means of establishing dosing parameters for cancer patients the cryopreservation process significantly affected the level of $CD34^+$ expressing cells in PBMC samples. de Boer et al. [61] have also reported the impairment of function in $CD34^+$ cells which resulted in a reduction in the effectiveness of stem cell graft procedures. Reports detailing similar reduction/losses in post-thaw functionality in gametes have also been described [62, 63]. Studies on the cryopreservation of spermatozoa have now linked molecular based stress responses and the loss of acrosomal and motility functions. Other studies have now associated negative effects of cryopreservation on the impairment of biochemical functionality in hepatocytes [22, 64] and cardiomyocytes [57]. These studies have helped to further our understanding and increase our recognition of the downstream effects cryopreservation may have on cellular function.

4.8 Cryopreservation Induced Cell Death

Biobanks experience a difficult, if not intractable, situation when faced with changes in cryopreservation protocols. While a number of organizational best practices exist (i.e. NIH NCI, ISBER, etc.), most focus on biobank management. Few effectively address process changes necessary improve cell functionality [2, 3]. Further, despite intensive research focused on improving cell preservation, not all mammalian cells cryopreserve "equally." To highlight this issue, Lane [5] stated that "*Few scientific problems have proved as intractable as cryopreservation*" and "*…cryobiology has been straitjacketed by its need to conform to the intractable laws of biophysics. For all its successes*, *cryobiology has been stuck in a rut*." Further, Mazur [65] has stated that "*The problem today* (with cryopreservation) *is that applying basic principles of biophysics simply cannot solve many of the remaining challenges in cryobiology*." As traditional approaches to cell storage are applied to non-terminally differentiated mammalian cells, many of these native and engineered cell types prove refractory to cryopreservation. As described, even in "successfully preserved" cell systems, significant death (30–70 %) is often observed within 24–48 h post-thaw [20]. Structural protection is afforded to these cells, but mitigation of the preservation-induced stress response resulting in cell death many hours post-thaw remains a critical issue. As such, it is often the case that today's cryopreservation protocols provide effective strategies for structural preservation of most mammalian cell types but lacked to the molecular-based tools necessary to understand and mitigate much of the post-thaw damage. Multiple modes of cell death are recognized as contributors to cryopreservation failure.

4.8.1 Physical Cell Rupture

During the freezing process, solute is concentrated from approximately 350 mosmol to upwards of 10,000 mosmol [12, 29]. Cells exposed to these conditions will shrink severely but not necessarily experience a lethal event. During the post-freeze thaw, many cells will be subject to significant cell membrane damage resulting in rupture while other cells may experience membrane damage that is repairable. Not all cells respond the same as cell rupture may occur over many hours. The majority of membrane rupture occurs within minutes after thawing. Those cells rupturing one or more hours post-thaw experience non-repairable membrane damage and typically die through necrosis.

4.8.2 Necrosis

While ice-related rupture has been the primary focus of cryopreservation, necrotic cell death has also been observed in numerous cases of cryopreservation failure [17, 23, 66]. Necrosis is an energy independent form of cell death characterized by the swelling of a cell and its constituent organelles, loss of membrane integrity, lysosomal rupture, random DNA fragmentation by endonucleases and ultimately cell lysis [67–70]. As a result of cell rupture and the associated release of cytokines, there is typically an activation of an immune and inflammatory response *in vivo* [67, 68, 70]. The progression of necrosis often occurs rapidly in a matter of minutes to hours. Induction is typically seen in a response to severe cellular stress and results in the activation of detrimental intracellular signaling cascades. Necrotic cell death has been reported to be activated by stressors such as ischemia, osmotic shock, severe thermal stress, ionic dysregulation, toxic agents, etc. Interestingly, many of these necrotic activating stressors are also involved in or associated with hypothermic storage and cryopreservation.

4.8.3 Apoptosis

Apoptosis is a form of gene regulated cell death often referred to as programmed cell death. It differs from necrosis in that it is an energy-dependent process characterized by cell shrinkage, chromatin condensation, intact membranes but with phosphatidyl serine inversion, non-random DNA cleavage, and the formation of organelle containing "blebs" [67–72]. Unlike necrosis, apoptosis does not elicit an immune response *in vivo* but instead cells shed the apoptotic blebs which recycle cellular materials through phagocytosis. Apoptosis is induced by a number of different stressors that can specifically initiate the apoptotic response in the mitochondria, the plasma membrane or the nucleus [71–73]. Apoptosis can be induced by starvation (nutrient deprivation), temperature changes, viral infection, hypoxia, radiation, toxic compounds, osmotic stress and many other stresses. There are two canonical "branches" of apoptosis which have been identified in cryopreservation failure: the extrinsic or membrane-mediated and the intrinsic or mitochondrial-mediated pathways. Additionally, studies show that cross-talk, feedback and amplification pathways exist [33, 34, 74]. The identification of a third, nuclear-mediated apoptotic pathway further complicates a complete delineation of the cryopreservation process.

4.8.4 Necroptosis

As ongoing cell death research has continued to elucidate the specific biochemical mechanisms that trigger and propagate programmed cell death pathways, an alternative form of cell death has been identified [75]. Given the name necroptosis, this recently identified type of cell death has been shown to result in a necrotic-like execution with classical hallmarks such as cell swelling and membrane lysis while remaining highly regulated which distinguishes it from the conventional definition of necrosis. Research has now begun to reveal the distinct mechanism of action responsible for the activation of this pathway. Specifically, it has been shown that this mode of programmed necrosis is triggered through the signaling of death receptors, such as tumor necrosis factor receptor 1 [76]. The binding of the respective ligand (TNF-α) to the death receptor, similar to membrane-mediated apoptosis, results in the recruitment of intracellular signaling proteins and in turn the formation of an active complex responsible for downstream effects. Central to this necrotic complex is the kinase activity of receptor interacting proteins 1 and 3 (RIP1 and RIP3, respectively) and their substrate, the pseudokinase mixed lineage kinase domain-like protein (MLKL) as the core machinery for execution [77, 78]. Continued efforts will be necessary to further clarify the specific signaling cascade of necroptosis and how exactly the apoptotic/necroptotic balance is controlled by the cell during programmed death. However, the role of necroptosis in particular disease states and other biopreservation related stress conditions such as ischemia/reperfusion injury is becoming more

evident [79, 80]. As such, efforts to understand the complex cell death interplay at the molecular level will be paramount for improving future biopreservation endeavors.

4.8.5 Transitional Cell Death

Molecular-based cell death is typically thought to proceed through either an apoptotic or necrotic pathway. Apoptosis has been viewed as a "true organized molecular response" with necrosis considered "to involve random molecular events" at the intracellular signaling level. While accurate, the cell death landscape has evolved substantially over the last decade to suggest that classical apoptosis and necrosis represent more extremes on a continuum of molecular-based cell death [4]. Apoptosis is now considered to be a mode of cell death that can present in several forms including (a) Type I, the conventional view of apoptosis, not involving lysosomes but relying on caspase activation, (b) Type II, by contrast, is characterized by lysosomal-linked autophagocytosis, and (c) Type III, lysosomal-independent, necrosis-like apoptosis characterized by swelling of intracellular organelles [73]. It is now known that a cell's commitment to death causes an apoptotic activation and progression to cellular execution (type I classical apoptosis) or to a point where the stress becomes too great or energy reserves (ATP levels) too low resulting in a shunting from apoptosis to necrosis (secondary necrosis) [25, 30, 74, 79, 80].

Transitional cell death has been demonstrated in a number of studies, including some in cryopreservation, and has provided a basis for the cell death continuum concept emphasized here. Common stressors such as nutrient deprivation, DNA damage, cytokine exposure, cytotoxic agents, oxygen deprivation, ionic imbalance, etc. have been shown to result in the activation of both apoptosis and necrosis in a multiplicity of cell systems. The determination of apoptotic or necrotic activation is believed to be based on the relative degree of the stress experienced by the cell. The transitional nature of the cell death pathways in response to similar stressors creates a difficult environment to characterize. This is especially true as it applies to situations where multiple stressors are involved, such as cryopreservation.

4.9 Re-optimization of Cryopreservation

4.9.1 Initiation of Cryopreservation-Induced Molecular Death

It is now clear that much of the cell death associated with cryopreservation is linked to the execution of molecular-based cell death cascades [25]. However, limited detailed investigations into the initiating stresses have been reported. As described, the cryopreservation process exposes cells to stressors, many of which can initiate a molecular death response [3, 25, 37]. These factors include metabolic uncoupling, production of free radicals, alterations in cell membrane structure and fluidity, dysregulation of cellular ionic balances, release of calcium from intracellular stores, osmotic fluxes, and cryoprotective agent toxicity. This listing of stresses is by no means complete, but serves as a guide to the complexity of the stress response and multiplicity of potential cellular initiation sites. In an effort to provide insight into the effect of the various stressors associated with cryopreservation, studies have begun to focus on potential initiation sites of apoptosis within a cell. These studies remain in limited but nonetheless shed light onto the role of various pathways of molecular cell death, including the cell membrane, nucleus, and mitochondria associated with low temperature exposure.

4.9.2 Management of Cell Death

Decades of cryobiological research have yielded numerous cell preservation protocols based almost exclusively on one facet of the cold chain – osmometric parameters determinant of water flux as interpreted by changes in cell volume. This information is crucial to successful preservation but only part of the story. The physi-

ological responses of cell stress may direct many cells, especially those undergoing mitosis toward cell death. These molecular responses launch early stage apoptosis during pre-freeze incubation with cryoprotective media. During this period metabolic dysregulation results in free radical production [35, 81], cellular acidosis [28], protein unfolding [82–85] and ion imbalances. These stressors continue to strengthen during the ice growth phase and into the glass transition temperature range. This set of events is also manifest if an extracellular vitrification strategy is employed. Extracellular ice, while participatory in exacerbating stress buildup, is not a defining factor in cell death if "optimal" levels of cryoprotectants are uses. Cell then enter as dormant period but with various cell death pathways activated and primed for execution upon thawing [17, 18, 20, 25].

Differences in the sensitivity of various cell types to cryopreservation processes are well known. In an article by Van Buskirk et al., [30] it was suggested that the basis for differing cellular survival is linked to individual cell stress response and the resultant differential activation of cell death processes. The discovery of molecular responses in cells to the preservation process has therefore resulted in a variety of attempts to control these events in an effort to improve outcome. These attempts have included alteration in solution design (cryoprotectant carrier media), addition of cryoprotective agent cocktails, and the incorporation of select compounds for the Targeted Control of Apoptosis (TAC) during the cryopreservation process.

4.9.3 Carrier Media

The mitigation of the molecular-based stress responses to low temperature exposure and storage has been shown to be attainable with cryopreservation solution formulation that addresses both physical and cellular related events [4]. The concept of specialty preservation media has evolved out of the organ preservation specialties. The Belzer and Southard team [86, 87] first developed ViaSpan® (the University of Wisconsin solution) to support the transport of organs (pancreas, kidney and liver). ViaSpan®, formulated for hypothermic storage, was the first solution designed to manage select putative stress factors and became the first "intracellular-like" preservation medium. In the decade that followed additional preservation solutions were developed (i.e., Celsior, HTK – Custodiol, HypoThermosol, Unisol, and others) [28]. More recently, cryopreservation solution formulation has moved beyond the addition of a penetrating cryoprotective agent such as DMSO (5–15 %) to cell culture media, buffered saline or these media plus serum or a protein component [37]. Now recognized as essential to optimization of the cryopreservation process is the maintenance of proper cold-dependent ion ratios, control of pH at lowered temperature, prevention of the formation of free radicals, oncontic balance, the supply of energy substitutes, etc. [25, 39] Traditional media fall short in addressing changes in solution pH, free radical production, energy deprivation, etc. Accordingly, the basal properties of these historical preservation media often do not provide for protection at the cellular level [37]. In attempt to address this issue, the cryopreservation sciences have taken lead from the organ preservation and molecular biology arenas combining these knowledge bases to increasing cell survival. Complex cryopreservation media (CCM) including Viaspan, CryoStor, Unisol, Adesta, Celsior, and others, to name a few, when combined with CPAs for have been reported to improve cell survival to varying degrees. Improvements have been observed in systems including hepatocytes [21, 22], cord blood stem cells [40], PBMC's [88, 89], fibroblasts [20], keratinocytes [90], blood vessels [91] and engineered tissues [92]. In these studies, evaluation of the cryopreservation media was conducted and correlated with improvements in cell survival, function and growth. The improvement was not noted immediately post-thaw but not until following manifestation of the molecular-based events was the effect observed. It is now recognized that the integration of an intracellular-type solution with a penetrating cryoprotectant along with an understanding of the molecular responses of the cell at low

temperature, provides for improved cryopreservation outcome [4, 37, 39]. The success of these solutions is linked to an in depth knowledge and understanding of the cell death pathways activated as a result of cryopreservation-induced cell stresses. To this end, studies have suggested that the improvement in cell survival and function was due to a reduction of both apoptosis and necrosis during post-thaw recovery although the mechanism of which remains unknown [20, 25, 93, 94].

4.9.4 Target Control of Apoptosis

In an effort to mitigate the pro-death cascades activated as a result of both the pre-storage processing and subsequent preservation steps, a number of different targeted approaches have been taken. Initial strategies for targeted control utilized broad acting stress reduction agents such as free radical scavengers, antioxidants, protease inhibitors and ion chelators as additives to cryopreservation and hypothermic storage media as a means of inhibiting cell death [4, 81, 88, 92, 93, 95–98]. Continued efforts in this approach began to use more specific molecular-based agents to precisely target proteins and cell stress pathways. Specifically, with the use of caspase inhibitors to target pro-apoptotic signaling, studies demonstrated improved biopreservation of numerous different cell systems [17, 18, 88, 92, 93, 98]. Interestingly these improvements were observed as a decrease in both apoptosis and necrosis, again demonstrating the complex interplay of the cell death continuum [25, 36, 84, 88]. More recently, the pro-apoptotic protein Rho-associated kinase (ROCK) has been successfully targeted through the use of a ROCK inhibitor. This finding has been critical for the field of stem cell biology as the use of ROCK inhibitor, both during and post-thaw, has allowed for the successful dissociation and cryopreservation of embryonic stem cells as it has increased post-freeze survival and decreased spontaneous differentiation that resulted from preservation related stress [99, 100]. Additional research has begun to demonstrate improved biopreservation outcomes through the control of cell stress sensing and response elements that lie upstream of the caspase execution pathway. One such pathway that has garnered interest in this respect in the role of endoplasmic reticulum stress and the subsequent triggering of the Unfolded Protein Response (UPR) as a component preservation-induced cell death. Reports have detailed the important and differential role that the UPR pathway has in biopreservation outcome [83–85] and that a more complete understanding and control of this complex signaling will be necessary for next level preservation advances.

4.10 Summary

The cryopreservation of biologics such as cells and organs, has relied on low temperature to provide “on demand” access. While today’s standard of practice for cryopreservation still focuses primarily on the control of osmotic flux, ice formation and associated stressed, numerous reports have emerged over the last decade demonstrating the critical role of molecular-based stress response pathways and their control plays in cryopreservation outcome. The impact of this molecular aspect extends well beyond influencing cell death but also has a long term impact on biochemical pathways and cellular functionality post-thaw. As such, the ability for today’s biobanks to provide the highest quality samples to enable future discovery will depend on a paradigm shift in cryopreservation strategy. This shift must recognizes that (a) structural methodologies are reasonably effective (“optimized”) in preventing ice-related damage and (b) there remains a compelling need to decipher the cell’s responsiveness to the sever oxidative stressors attendant to a freeze-thaw excursion is required to overcome the significant cell death after thawing [4, 20].

Beginning with the initial step of cell processing (i.e. lifting cells in culture or surgical tissue excision), oxidative stresses parameters begin to compromise the normal physiology the cell. An initial element of any cold chain optimization strategy is the standardization of the isolation

steps (time, temperature and immersion media) and the addition anti-stress agents (i.e. free radical scavengers, molecular-based cell death blockers, buffers operative at low temperatures and oncotic agents to mitigate cell swelling). Cell death cascades that "play out" post-thaw are triggered (sensitized) during pre-freeze manipulation. The suppression of cryopreservation-induced molecular-based cell death can further be accomplished by a number of other strategies either individually or in combination. These include the utilization of (a) complex cryopreservation media (with an intracellular-like ion distribution, appropriate organic buffer and impermeants to protect against osmotic extremes), (b) various free radical scavengers, (c) Targeted Apoptotic Control strategies (apoptotic inhibitors). The timing of the addition of a complex cryopreservation media (CCM) may be cell type specific [4, 30, 37, 39]. While the majority of these efforts focus on the pre-freeze and freezing portion of the cryopreservation process, a similar TAC-based strategy can be employed following a rapid thaw to samples currently banked utilizing today's standard of practice protocols. While not as effective as front end intervention in the process, post-thaw manipulation strategies offer the potential to salvage cell populations enhancing downstream utilization through improving overall survival and/or cell function.

As the literature clearly demonstrates, a shift to molecular-based cryopreservation strategies can provide for improved outcome, it is important to recognize that each of the "optimized" cryopreservation protocols established over the past five decades are not likely to remain optimal with the incorporation of a CCM or other biochemical stress control strategy during or following freezing [17, 18, 20, 22, 39, 88]. This suggests that many if not all of the stages of the cold chain associated with cryopreservation may warrant re-investigation in the future. For example, nominal cooling rates of 1 °C/min are commonly applied despite reports indicating that higher cooling rates are beneficial with varying CPA concentration and types are used [51, 101–103]. In this regard, the use of a CCM with traditional levels of CPAs ought to support the use of higher cooling rates. Further, some cells survive well at cooling rates up to hundreds of degrees per minute. Similarly, thawing rates and the manner in which thawing is applied warrants continued study. Thawing should be as rapid as possible but, as previously discussed, with a methodological approach that supports a uniform thaw and is reproducible.

It is without doubt that the discovery of a complex molecular response of cells and the influence of cryopreservation-induced cell death on overall cell survival and function has had tremendous impact on cryopreservation research over the last 10–15 years. While recognized, these new principles and practices have yet to be implemented into biobanking strategies or even into mainstream discussion in the biobanking community. As the demand for the highest quality frozen cell products continue in support of growth in areas such as discovery science, diagnostics, stem cell biology and personalized medicine, the biobanking industry is now faced with the immediate challenge to embrace and incorporate improved strategies and protocols, extending beyond those focused on management and best practice protocol standardization, which address the molecular biological control/preservation aspect of cells during the cryopreservation process.

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Biobanking for Personalized Medicine

5

Angen Liu and Kai Pollard

Abstract

A biobank is an entity that collects, processes, stores, and distributes biospecimens and relevant data for use in basic, translational, and clinical research. Biobanking of high-quality human biospecimens such as tissue, blood and other bodily fluids along with associated patient clinical information provides a fundamental scientific infrastructure for personalized medicine. Identification of biomarkers that are specifically associated with particular medical conditions such as cancer, cardiovascular disease and neurological disorders are useful for early detection, prevention, and treatment of the diseases. The ability to determine individual tumor biomarkers and to use those biomarkers for disease diagnosis, prognosis and prediction of response to therapy is having a very significant impact on personalized medicine and is rapidly changing the way clinical care is conducted. As a critical requirement for personalized medicine is the availability of a large collection of patient samples with well annotated patient clinical and pathological data, biobanks thus play an important role in personalized medicine advancement. The goal of this chapter is to explore the role of biobanks in personalized medicine and discuss specific needs regarding biobank development for translational and clinical research, especially for personalized medicine advancement.

Keywords

Biobank • Biospecimen • Personalized medicine • Cancer • Pathology

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5.1 Introduction

Identification of biomarkers in patient blood or tissues that are specifically associated with particular medical conditions such as cancer, cardiovascular disease and neurological disorders are useful for early detection, prevention, and treatment of the diseases. Availability of biomarkers for disease diagnosis and prediction of patient prognosis and therapy promises personalized medicine. Patients are selected based on the presence of particular gene mutations in their samples can receive personalized treatment. In non-small cell lung cancer, for example, an EGFR mutation test is performed to determine if the EGFR mutation exists in the patient's tumor sample. If present the patient will receive a personalized treatment with EGFR tyrosine kinase inhibitors such as gefitinib and erlotinib [1, 2], whereas melanoma patients carrying a BRAF V600E mutation are treated with BRAF inhibitor vemurafenib [3]. Personalized medicine delivers the most appropriate therapy to individual patients with minimal toxicity and depends on readily available, high-quality and well annotated human biospecimens.

Human biospecimens include tissue, blood, urine, bone marrow and other bodily fluids which are a source of germline DNA, RNA, proteins and other metabolites that are required for genomic research and biomarker development. Banking human biospecimens and associated patient clinical data allows future translational research for the development of therapies and biomarkers, which is necessary to enhance personalized medicine. In order to provide a foundation for personalized medicine, biobanks should be sufficiently large to obtain reliable results through larger studies. Large scale efforts, such as The Cancer Genome Atlas (TCGA) project have successfully performed profiling studies of many cancer specimens to help catalog the spectrum of mutations present in different tumor types including ovarian cancer, breast cancer, colorectal cancer, lung squamous cell carcinoma, and clear cell renal cell carcinoma (http://cancergenome.nih.gov/) [4–8]. In addition, by banking the biospecimens it is possible that as new technologies are implemented, new assays performed on the banked biospecimens will provide additional insight in the future, especially nowadays with next generation sequencing providing a powerful tool to sequence the whole genome in an efficient and inexpensive way.

Proper collection, processing, storage, and tracking of biospecimens are critical components for biobank operations. The workflow of biobanking begins with obtaining the informed consent from participants then collecting specimens. Tissue specimens can be collected from patients who receive surgery or a biopsy through pathology. Other bodily fluids such as urine, blood and saliva can be collected through clinical care. After collection the biospecimens must be processed, aliquotted and frozen in containers suitable for long-term cryopreservation. Patient clinical information must be stored in a secure database that can be linked with the samples in the biobank. Finally, distribution of biospecimens and patient data must be in compliance with ethical regulations. The practicalities of biobanking are highly complex in their operation including many technical, legal, ethical, and other issues and require significant collaborative efforts [9, 10]. Expertise in standardization and quality control, information technology, laws and regulations, and clinical and pathological knowledge are generally required for biobank operation and management. A biobank that supports personalized medicine involves patient care and would require the highest standards for operations, and rigorous quality assurance and quality control. As human biospecimens are sequenced and actionable results will be returned to clinical practices, these types of biobanks should be considered as clinical biobanks and certification such as the College of American Pathologists (CAP) accreditation would be required [11].

5.2 Biospecimen Procurement and Processing

Biospecimens should be procured from patients with informed consent. The informed consent provides a description of the biobank project and

research study includes an explanation of all procedures, the participant's role, description of benefits, reasonably foreseeable risks, and relays that participation is voluntary [12]. Addressing the need to obtain patient informed consent is a major consideration when developing a biobank. Federal regulations such as Common Rule and Health Insurance Portability and Accountability Act (HIPPA) mandate that consent forms be written in plain language that the subject can understand [13, 14]. Broad consent allows biospecimens to be banked in a biobank for unspecified future usage, which is more of a general consent procedure and benefits the biobank's long term goals [15]. With the broad consent, two institutional review board (IRB) approved protocols are generally required: a biospecimen collection protocol for biobanking and a study protocol for the request and use of the biospecimens. Specific information should be included in the consent form as to whether biobank participants could be contacted again in the future, whether the biospecimen could be shared with outside researchers who are not part of the original institution where the biospecimens were procured, and whether the biospecimens could be used for genetic testing, etc. In addition, as new high throughput technology such as next generation sequencing is emerging, patient consents should describe the procedures for handling incidental findings as well.

Biospecimens include tissue, blood, urine, bone marrow and other bodily fluids. Solid tissues obtained from routine clinical biopsies or surgical procedures should be collected by or under supervision of a pathologist. The pathologist plays an essential role in determining tissue allocation for diagnosis and for biobanking. Specifically, when solid tumor was removed from a patient, careful gross examination should be done by a pathologist or pathologist's assistant. Essential portions for dissection are determined by the pathologist for routine diagnosis, and additional portions of tissue can be dissected and collected for biobanking without compromising routine pathological diagnosis. Sometimes tissue allocated for biobanking may need to remain in the pathology lab until the final diagnosis has been made.

High speed collection and efficient processing are essential for high quality tissue biobanking. The efficiency of the transportation of tissue biospecimens from the operating room to the pathology lab, pathological pre-procurement evaluation, and tissue processing are key steps in maintaining the quality of procured tissue samples. A large body of research has demonstrated that prolonged ex-vivo ischemia time can compromise biospecimen quality on RNA and protein level and could potentially impact the research results [16, 17]. Therefore, once tissue is removed from the patient, gross examination and tissue processing by the pathologist and biobank staff should be completed as soon as possible, ideally within 30 min to avoid prolonged ex-vivo ischemia time on the tissue sample. In addition to tissue biomolecular quality, subsequent morphological analysis of frozen sections of biobanked tissues by a pathologist is generally required to evaluate the tissue histological quality. This procedure includes confirming pathological diagnosis, tissue heterogeneity, tissue scoring, present or absence of tumor cells, inflammatory and necrotic areas, etc. Pathological analysis of biobanked tissue should be correlated with original pathological diagnosis of the patient. When a discrepancy between the original pathological diagnosis and biobanked tissue diagnosis occur, the banked frozen tissue may be released for subsequent diagnostic analysis to make sure the routine diagnosis get done properly. Fresh frozen tissue collected for biobanking should be aliquotted, snap frozen, and stored in −80 °C or liquid nitrogen freezers to avoid repeated freeze thaw cycles and keep the biospecimen integrity. Formalin-fixed, paraffin embedded (FFPE) tissue blocks may also be collected for biobanking but the corresponding H&E slides are required to be examined by a pathologist to determine the area and amount of tumor or diseased tissue to be collected for biobanking.

In addition to tissue, blood is one of the most easily accessible and widely used biospecimen types in clinical and translational research. Blood collection and storage are less complex than tissue biobanking. Collected blood samples should be fractionated into plasma, serum, buffy coat and red blood cells and stored separately to

maximize the value of the sample. Depending on downstream research purposes, plasma and buffy coat are collected in different anticoagulant coated collection tubes such as ethylenediamine tetraacetate (EDTA), citrate, or heparin tubes. EDTA coated collection tubes are suitable for a wide range of DNA and protein based arrays [18], while plasma from heparin-stabilized blood is often used for metabolomic studies [18, 19]. Citrate produces a higher yield of lymphocytes, therefore citrate dextrose coated tubes are used for harvest of peripheral blood lymphocytes [18, 20]. Buffy coat can be used as a long term biobank specimen for DNA and RNA in lieu of immediately isolating the nucleic acid at the time of blood collection. Studies have shown that germline DNA extracted from buffy coat can be used widely for biomarker studies [21, 22]. Serum is collected in a tube containing a clot accelerator such as silica and thrombin and is useful for certain assays in clinical biochemistry and metabolomic studies [18]. The UK biobank has a comprehensive blood collection program and collects blood in all different types of anticoagulant coated tubes and serum-separating tubes [18]. In addition, cancer-derived materials in the blood such as circulating tumor cells and cell-free circulating tumor DNA are becoming highly sought-after biospecimen types. Circulating tumor cells are disseminated from primary and/or metastatic tumors throughout the circulatory system while fragments of DNA are shed into the bloodstream from dying cells [23]. Circulating tumor cells and cell free circulating tumor DNA can be isolated and used to follow disease progression. Therefore, biobanking of such blood-based materials provides a very useful resource to develop personalized therapies. Urine is another easily obtained biospecimen and has been widely used for studies involving proteins, nucleic acids, or cells. Urine can be self-collected fresh in a clean container or may be collected from a catheter if patient already has a urine catheter. After collection, urine should be aliquotted and immediately frozen as whole urine. Some aliquots can be centrifuged and then the pellet and supernatant stored separately at −80 °C or in liquid nitrogen vapor. In addition, saliva, cerebrospinal fluid, pleural and abdominal fluids, gastric lavage, buccal smears, and Pap smears are also valuable materials to be considered for collection and biobanking. Such bodily fluids can be aliquotted intact or the cellular content can be enriched by centrifugation and storing the cell pellet separately from the clear supernatant. Similar to tissue biospecimens, bodily fluid specimens should be processed as soon as possible after collection and stored at −80 °C or in liquid nitrogen vapor for long term cryopreservation. The Early Detection Research Network (EDRN) standard operating procedures for collection of plasma and serum recommended that plasma EDTA tubes should be processed immediately or held for no more than 4 h at 4 °C prior to processing, and serum tubes should be allowed 30–60 min to clot, then processed in a centrifuge or held at 4 °C for no more than hours [24]. As new analytic technologies have evolved, biospecimen types and collection methodologies will continue to be developed as well.

5.3 Biospecimens Storage and Distribution

Although certain types of biospecimens such as FFPE tissue blocks and sections can be stored at room temperature, most biorepositories bank fresh frozen biospecimens to retain a high degree of nucleic acid and protein integrity. Therefore, biospecimens are typically stored at −80 °C or in liquid nitrogen vapor, and should be maintained in good condition until needed for experiment or analysis. Solid tissue specimens are preserved in a different way. Tissues are snap frozen with liquid nitrogen or embedded in optimal cutting temperature (OCT) medium then snap frozen and stored at −80 °C or in liquid nitrogen vapor for long term cryopreservation. FFPE tissue and RNAlater or PAXgene preserved tissue can be stored at room temperature for short term storage until usage, but should be stored at −20 °C or colder for long term biobanking. Several studies have shown that repeated freezing and thawing can lead to decreased RNA integrity in tissue and blood and significant change serum and plasma

proteomes [25–28]. Therefore, frozen biospecimens should be stored in several aliquots to avoid repeated freeze-thaw cycles. There is very little information available as to whether the storage of biospecimens in liquid nitrogen is better than storage at −80 °C. However, some studies showed that RNA integrity was reduced in specimens stored at −70 or −80 °C for over 5 years [29, 30], although no degradation of protein was seen in plasma stored at −70 °C for up to 59 months [31]. If possible, sample aliquots from the same patient should be stored in two separate locations (−80 °C and liquid nitrogen vapor) in order to protect the biobank from loss and maintain the quality of the biospecimens. Biospecimens that are housed in −80 °C freezers should be used first. The liquid nitrogen vapor storage serves as a "back-up" that will be used when samples in the −80 °C freezer have been exhausted.

Screw-cap cryovials are typically used for biospecimen containers for long term low temperature storage. The correct labeling of cryovials contain sample aliquots is important to ensure correct identification of the biospecimens. Barcoded labels can link the biospecimens to data recorded in the database and provide the most efficient labeling method. Cryovials that are stored in −80 °C and liquid nitrogen should labeled with temperature-resistant cryogenic storage barcoded labels to prevent cracking, peeling or degradation. Biospecimen ID, sample type, freezer location, and patient demographic and clinical data associated with the biospecimens should be centrally stored on a secure computer-based database system and should be backed up frequently. The available best practices for biorepositories such as International Society for Biological and Environmental Repositories (ISBER) best practices for repository and NCI best practices for biospecimen resource serve as guidelines and references for local standard operating procedure development [32, 33]. More recently, the College of American Pathologists (CAP) developed the standardization of biospecimen management which is the CAP accreditation for biorepositories program. The CAP biorepository accreditation checklist provides detailed accreditation requirements to ensure that standard operating procedures and quality assurance and quality control programs are implemented in the biorepository. Accreditation by the CAP focuses on quality, accuracy, and procedural consistency, which would help to significantly increase the value and quality of a biobank and maintain the highest standards of biobank operation. Biobanking in support of personalized medicine, which involves direct patient care, would require such a certification process to meet a recognized standard.

The infrastructure and accessibility of the biobank has a direct impact on personalized medicine. In order to have reliable access to high quality biospecimens with relevant patient clinical information, an easily accessible method should be generated to coordinate discovery and request of annotated biospecimens. Information technology plays an important role for biorepository data management and biospecimen accessibility. The main functions of biorepository information management systems are to track biospecimen acquisitions, processing, storage and distributions, manage patient information, and provide the ability to perform custom biospecimen queries. Patient demographic data, pathological diagnosis, biospecimen type and freezer location are generally essential data elements in the database. Certain laboratory data, e.g., ER, PR and Her2neu status for breast cancer, EGFR mutation status for non-small lung cancer, and BRAF mutation status for melanoma, and follow up outcome data is becoming more and more important as well and should be captured and stored in a central biospecimen database. An accurate biospecimen inventory and pertinent biospecimen-associated clinical data can significantly reduce the often overwhelming tasks of finding and retrieving biospecimens and increase the value of banked biospecimens. A searchable web-based tool to coordinate the request of annotated biospecimens will make the biobank more easily accessible [34, 35]. To protect patient privacy and confidentiality, the biobank should serve as an honest broker storing all data on a password protected computer, with support from an institutional secure server [34, 36]. The release or disclosure of protected health

information to any other person or entity will consist of only a limited data set application complete with a data use agreement as approved by the IRB and may also require an explicit consent of the patient. All released data should have a release code, e.g., biospecimen reference number that allows the biobank personnel to re-link the data to the sample and to trace the data's origin.

Biobank operations should receive guidance from a multidisciplinary advisory committee. The committee should consist of faculty members who represent surgery, pathology, bench science, clinical science, biostatistics, bioethicists, and other appropriate fields. The advisory committee serves to guide polices, reviews biospecimen requests to assure their scientific merit, and prioritizes resources to enable fair access to valuable limited biospecimens. Biobanking takes years to develop for biospecimen collection and follow-up clinical data capture and requires adequate staffing, including pathology support, management, and technical personnel. The infrastructure investment and operating cost of a biobank is a significant commitment, and building and operating even a small biobank is costly [37, 38]. Therefore, long-term institutional support and chargeback mechanisms should be in place in order to make long term sustainability of the biobank. Since biobanks that support personalized medicine involve direct patient care, reimbursement by patient health insurance companies may ultimately be required and certain accreditation may be necessary for these types of biobanks [39].

5.4 Quality Assurance and Quality Control

The most important aspect of a successful biobank for personalized medicine is the quality of the banked biospecimens. Studies using low quality biospecimens will likely generate erroneous and misleading data. Quality assurance and quality control is of utmost importance for biobank operation. Biobanks should have a documented quality management program and detailed SOPs to ensure the quality of biobank services and performance. SOPs should be prepared in detail so that biobank personnel can easily follow and accurately perform the methods therein, and a process for periodic assessment of the quality of banked biospecimens should be in place. As recent acceleration on new high throughput "omics" platform technology increase the demands on biospecimens at the DNA, RNA and protein level, banking fresh frozen biospecimens to retain a high degree of nucleic acid and protein integrity becomes more important. Therefore, proper biospecimen handling and identification is crucial for high quality biobank development and management.

Total RNA extracted from human biospecimens should be run on a spectrophotometer and a denaturing agarose gel. A ratio of spectrophotometric readings at 260 and 280 nm (A260:A280) greater than 1.8 indicates the acceptable purity of RNA [40]. For intact RNA, there are two distinct bands on the electrophoretic agarose gel corresponding to 28S and 18S ribosomal RNA. RNA integrity can be measured by the ratio of 28S to 18S ribosomal RNA (rRNA). A 28S/18S ratio of 2 or higher is considered as high quality RNA but generally a 28S:18S > 1.0 could be considered of good quality [41]. Since this approach is subjective and relies on gel image interpretation, a newly developed automated RNA quality measurement, the RNA Integrity Number (RIN), has been widely used [42, 43]. The RIN is generated by a software algorithm and is a user independent, automated and reliable procedure for standardization of RNA quality control [42]. More recently, the RIN^e was developed and designed for using on the Agilent TapeStation platform, but the values of RIN^e are equivalent to RIN values [44]. The RIN or RIN^e values range from 10 to 1, whereby the intact highest quality RNA is assigned a RIN or RIN^e value of 10. High quality RNA is characterized by two clear, well defined 28S and 18S peaks and little noise between the peaks and minimal low molecular weight noise before the 18S peak (Fig. 5.1). In general, a RIN of 5 or higher is considered as high quality RNA and suitable for RT-PCR analysis [45, 46]. RNA extracted from FFPE tissue or biospecimens that are preserved via different protocols such as

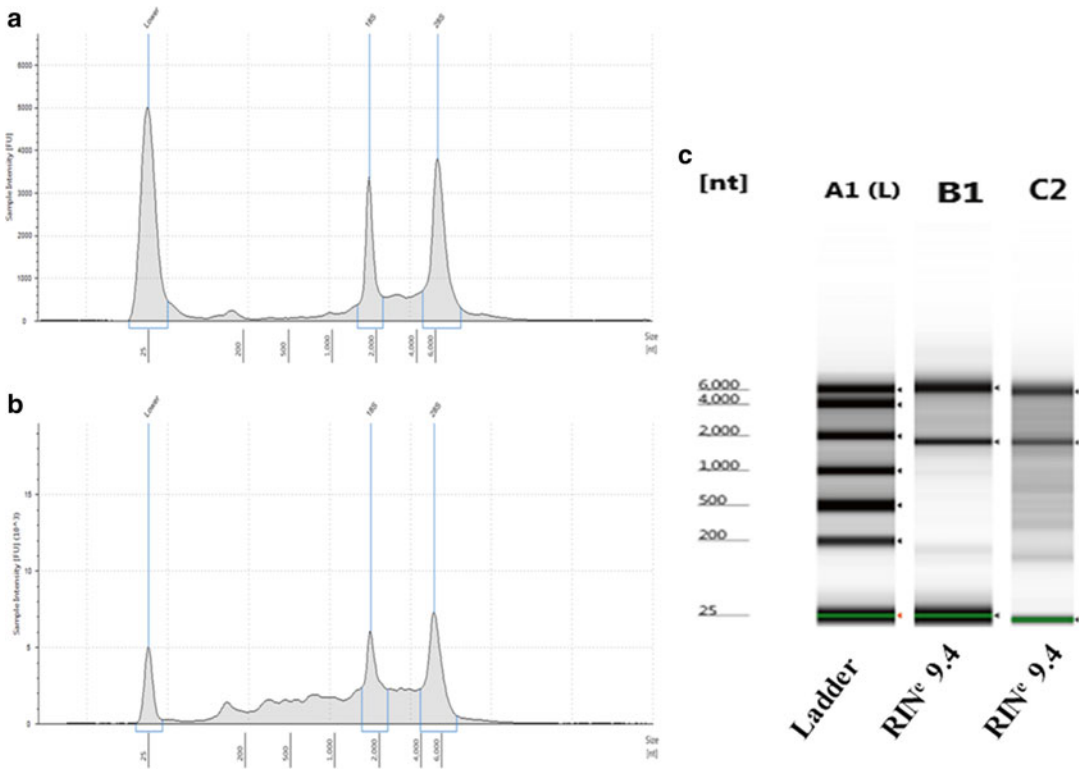

Fig. 5.1 RNA quality control. The quality of RNA that extracted from tissue and blood was analyzed using the Agilent TapeStation. (**a**) An example of high quality RNA shows the peaks of 18S and 28S rRNA are clearly visible with a high RIN^e number (RIN^e = 9.4), and very low noise between the 18S and 28S peaks. (**b**) The lower panel shows partially degraded RNA with a low RIN^e number (RIN^e = 5.9), and high noise between the two peaks and before 18S peak. (**c**) Gel image of the same RNA in **a** and **b**

alcohol fixation generally require rigorous quality assurance and quality control procedures. The use of the RIN or RIN^e value that generated by an Agilent Bioanalyzer or a TapeStation may not be good enough to assess the quality of RNA that is isolated from FFPE tissue. To accurately assess the quality of FFPE RNA, two sets of primers for the upstream end and downstream end of housekeeping genes such as beta-actin, are used for the real-time PCR analysis. A low ratio of the 3′ end to the 5′ end PCR products indicates high quality RNA [11].

DNA extracted from biobanked specimens can generally be assessed using spectrophotometric measurements such as the Nanodrop to determine the DNA quantitation and purity. Same as the RNA measurement, a ratio of spectrophotometric readings at 260 and 280 nm (A260:A280) greater than 1.8 indicates an acceptable purity of DNA. To access the quality of DNA, several sets of primers of housekeeping genes such as β-globin can be used to amplify different length fragments, and the maximum amplicon size positively correlates with DNA quality. More recently, the Agilent TapeStation was developed and provides both quantity and quality assessment of DNA in a single step [47]. Using a TapeStation, high quality DNA runs as a clearly defined single band while degraded DNA runs as a smear pattern with different sized fragment bands (Fig. 5.2). Integrity of the DNA and RNA extracted from buffy coat and other cell components of non-tissue biospecimens such as blood, blood fractions, urine, etc. can be measured in the same way to assess the non-tissue specimen quality.

Several different quantitative protein assays exist such as the bicinchoninic acid assay, Lowry

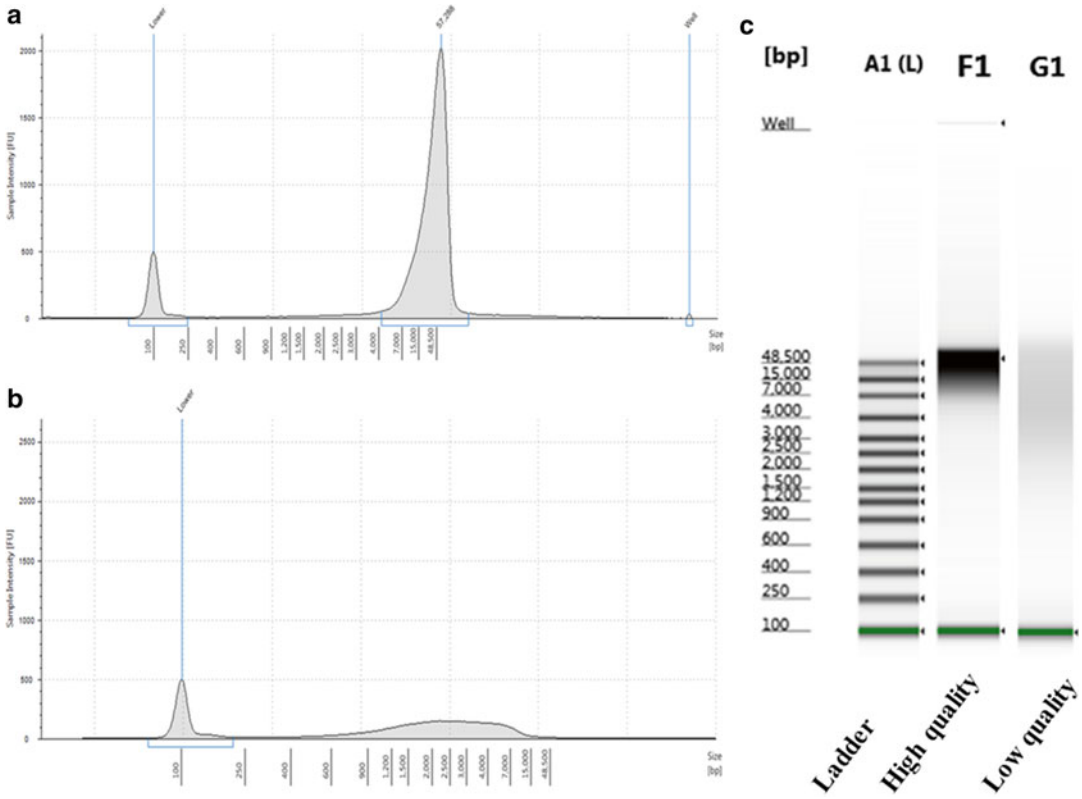

Fig. 5.2 DNA quality control. The quality of DNA that extracted from tissue and blood was analyzed using the Agilent TapeStation. (**a**) An example of high quality DNA profile generated by Agilent TapeStation shows a single large size DNA. (**b**) A profile of low quality of DNA was generated by the Agilent TapeStation shows smear pattern with DNA fragments. (**c**) Gel image of the same DNA in **a** and **b**

protein assay, and the Bradford protein assay for protein concentrations, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie Blue staining to determine proteome integrity. Specific antigens including those of phosphoproteins can be identified by Western blotting and immunohistochemical staining. Since these protein quality evaluation methods are usually not cost effective or efficient, it would be probably best left to end user investigators, depending on their specific application and analytical processes to perform their own protein quality evaluations. Similarly, the quality assurance and quality control of plasma, serum, and other bodily fluids primarily rely on investigator feedbacks. Documentation of selected parameters such as the time between sampling and freezing, the storage temperature, the duration of storage, and the acceptable number of freeze-thaw cycles would be important for bodily fluids quality assurance. Other laboratory activities such as documents control, records control, internal audits, and corrective and preventive actions are also important in the biobank quality management program.

5.5 Role of Pathologist in Biobanking

The role of the pathologist in biobanking has been well recognized [48–50]. Because of pathologist's role in examining human tissues for diagnosis or therapy of diseases, only pathologists understand which portion of tissue is needed for diagnosis and which portion of tissue can be biobanked. Since most solid cancer tissue specimens are composed of heterogeneous cell

types such as tumor, adjacent normal, stromal, inflammatory cell, etc., solid tumor tissues that are submitted to the biobank should be reviewed by a pathologist to determine the histological characteristics of the specimen. A recent study showed that tumor and adjacent normal tissue designated by the surgeon or pathology assistant clearly required the microscopic examination by a pathologist as tumor samples provided were mixtures of normal and tumor tissue, and normal tissue was in fact tumor [43]. Therefore, quality control of histologic tissue samples must be performed by a pathologist to review the morphology of tissue sections that are generated from banked biospecimens (Fig. 5.3a). The minimal quality control of solid tissue should be made on a mirror image section of tissue [51]. For certain types of tumor with an infiltrating pattern such as pancreatic and prostate cancer tissues, serial cryostat sectioning may be required for quality control purpose. A top section from OCT embedded frozen tissue block should be cut and stained with H&E for histologic examination. After several tissue sections are cut and submitted for nucleic acid extraction, a final section should be cut from the OCT block and stained with H&E to confirm the presence of tumor.

Histological quality control report should include verification of pathological diagnosis, disease status, evaluation of tumor purity, documentation of percentage of normal, stromal and necrotic tissue, and presence of inflammatory cells, etc. It is important to ensure that the tissue biospecimens (tumor or normal) stored in the biobank is exactly what is documented in the database. In addition, as the sensitivity of molecular biology technologies increases, varying results may be represented for dominant cell types but

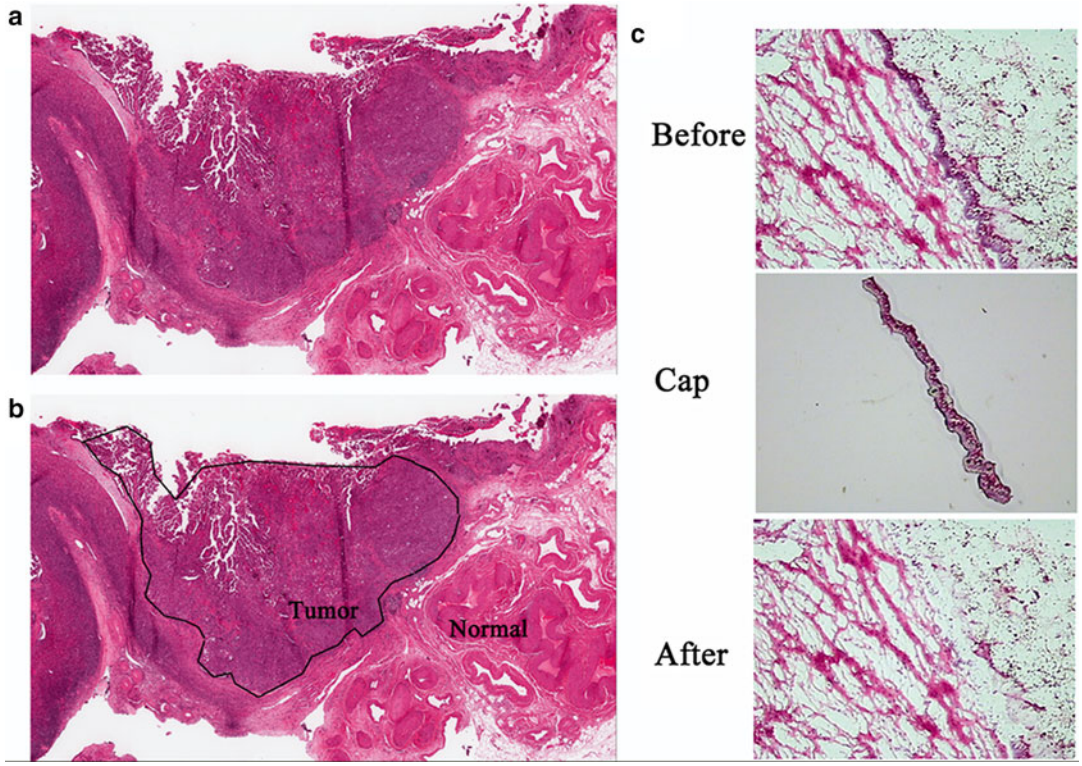

Fig. 5.3 Tissue histology quality control and optimization. (**a**) An example of ovarian tumor tissue H&E slide generated from OCT blocks. Pathological diagnosis was confirmed, and percentage of tumor content, normal and necrosis etc. was evaluated by a pathologist. (**b**) Tumor content was enriched using macrodissection prior to nucleic acid extraction. (**c**) An example of ovarian tumor tissue with less than 10 % tumor content was dissected using laser capture microdisection to enrich tumor content prior to nucleic acid extraction

not for the cell type of interest (e.g., tumor cell). Most solid tumor tissues are complex structures composed of heterogeneous mixtures of morphologically and functionally distinct cell types such as tumor, normal, stromal cells. To reduce heterogeneity within tissue, manual macrodissection or laser capture microdissection is often required to select specific cells of interest (e.g., tumor cells) to enrich the purity of specific cells prior to RNA/DNA/protein extraction for downstream analysis [52]. Both macrodissection and laser microdissection require a pathologist's expertise to recognize the specific cell type (tumor or normal cell) and mark the tumor and/or normal tissue for marcro- and/or micro-dissection to be performed by a biobank technician (Fig. 5.3b, c).

Tissue microarray (TMA) allows rapid and simultaneous morphological assessment in large sets of tissue specimens. Combined with immunohistochemical staining, in situ hybridization and fluorescent in situ hybridization (FISH) analysis, TMA provides a powerful tool for high throughput screening of molecular targets to facilitate the rapid translation of molecular discoveries to clinical application [53, 54]. Biobanked specimens are a huge resource for the construction of TMAs, thus a combination of TMA technology along with the availability of biobanked specimens can greatly help tumor profiling, biomarker identification, and validation. TMA blocks are prepared by transferring tumor tissues from many paraffin or OCT blocks to a single recipient TMA block. An H&E slide cut from the original paraffin or OCT blocks should be reviewed by a pathologist to identify the proper tumor foci for transferring to the recipient TMA block. Sections from TMA blocks can be stained with H&E and other special staining for simultaneous in situ analysis of multiple patient tumor samples by a pathologist (Fig. 5.4). In the era of personalized medicine, there is high demand for high quality, well clinically annotated cancer tissue for TMA analysis and other high throughput technologies such as genomic and proteomic analyses. The pathologist provides unique expertise and thus is a critical contributor in biobank operation and management.

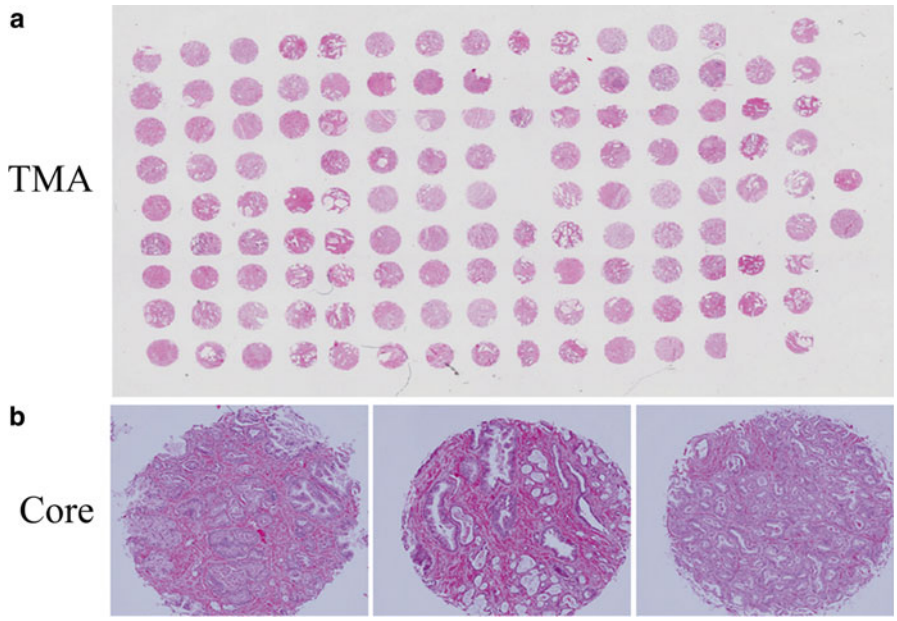

Fig. 5.4 Tissue microarray in biobank. (**a**) An example of prostate TMA block contains multiple patient tumor tissues. Section cut from TMA blocks was stained with H&E for morphological analysis by a pathologist prior to immunohistochemistry or in situ hybridization assay to enable the high throughput analysis of biomarkers in a large number of tumor tissue samples. (**b**) Representative cores of TMA

5.6 Whole Genome and Exome Sequencing

Innovative high throughput next generation sequencing is rapidly becoming the standard for molecular genetics assays. There is high expectation that the sequencing of the human whole genome and whole exome will ultimately revolutionize the practice of medicine. The ability to sequence each tumor's whole genome and whole exome quickly and inexpensively by next generation sequencing is changing the practice of oncology, which opens up new avenues in personalized medicine. Personalized treatment based on characterizing the individual patient samples by high throughput strategies has been attempted [55, 56]. The advances of next generation sequencing allow for the detection of informative mutations in multiple genes and pathways which, in turn, generates a comprehensive individual mutational landscape at an affordable cost, which tremendously facilitates the selection of targeted therapies. Next generation DNA and RNA sequencing undoubtedly requires well characterized, high quality human biospecimens [57]. Data generated by next generation sequencing using biobanked specimens should be stored in a secure central database for future research use. These sequencing data along with patient clinical information provide an invaluable data resource for new discovery without repeated biospecimen acquisitioning and thus reduce the costs of subsequent studies. The reuse of these data tremendously promises efficiency and effectiveness of translational and clinical research. However, future analysis of these data beyond the scope of the original study should be incorporated into the informed consent document and would need a separate IRB approval. To protect patient privacy and confidentiality, patient identifiers may need to be replaced with a computer generated barcode and a single link between sequencing data and patient clinical information should be maintained in a secure database. Whole genome and whole exome sequencing and biobanking are increasingly playing a critical role in identifying genetic variation and the associations between the genetic variation and drug efficacy and clinical outcomes, which provide a foundation and pave the way to advance personalized medicine.

5.7 Managing Incidental Findings

Next generation sequencing has the capacity to generate massive amounts of data that is often well beyond the original study question for ordering the sequencing. Incidental (or secondary) findings with potentially clinically relevant significance, but are not related to the original question, are increasing and unavoidable. Although there is an active debate about the return of incidental findings in genomic research, it is recommended that certain findings and results that reveal an established or substantial risk of serious health conditions, validated by a Clinical Laboratory Improvement Amendments (CLIA) certified lab, and are clinically actionable should be returned to consented biobank participants [58, 59]. The American College of Medical Genetics and Genomics (ACMG) recommends a minimum list of conditions, gene and variants for return of incidental findings in clinical sequencing, which include BRCA1 and BRCA2 for hereditary breast and ovarian cancer, APC for familial adenomatous polyposis, and RB1 for retinoblastoma, etc. [60]. More recently, the Presidential Commission for the Study of Bioethical Issues, a White House advisory panel recommended that researchers, clinicians, and direct-to-consumer firms that generate genomic, imaging, or other types of biomedical data should plan to encounter incidental findings and should develop a plan to communicate with patients, study participants, and consumers about how these findings will be handled [61]. Although the process of returning incidental findings to participants incurs significant costs to biobanks, biobanks in support of personalized medicine should strive to develop a responsible mechanism for returning the incidental findings. Particular procedures or policies, to determine how these findings will be handled, must be in place as personalized medicine continues to grow and evolve.

5.8 Conclusions

Personalized medicine has offered genuine hope for improved patient outcomes. Targeted therapies are beginning to make personalized medicine a reality in oncology. There are examples of highly effective drug treatments targeted to specific patient populations, such as the use of Herceptin in breast cancer patients who have Her2 over expression/amplification [62, 63], the use of Vemurafenib in melanoma patients who carry BRAF V600E mutation [62, 64], and the use of Xalkori in non-small lung cancer patients who carrying ALK mutations [65]. These biomarkers have enhanced the ability of oncologists to determine the best treatment with minimal toxicity. Biobanks play an important role for new biomarker discovery, which provide a foundation for development of novel personalized therapy. Biobank development and operation are quite complicated and require broad institutional cooperation and significant institutional supports. The required expertise in standardization and quality control, information technology, law and ethics governing biobank practices, and stakeholder communication and negotiation skills are necessary for high quality biobank development and operation. Biobanks that involve patient care by procuring and storing patient specimens or derivatives for clinically relevant molecular testing are an exciting movement, but require the highest standard of quality assurance, quality control, and necessary certification and accreditation. It is critical to have a large collection of biospecimens with patient clinical data for biomarkers to be effectively developed in a personalized medicine setting, and the pathologist should be involved in such biobank operation and management. The collection and quality assurance and quality control of biobanked tissue must be completed or supervised by a trained pathologist to ensure reliable and reproducible downstream molecular and genomic test results. As innovative high throughput next generation sequencing is rapidly becoming the standard for molecular genetics assays, biobanks in support of personalized medicine should strive to develop a responsible mechanism to handle the incidental findings. Ultimately, as personalized medicine expands, biobanks will be a necessary within medical centers to provide standard patient care.

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A Global View of Breast Tissue Banking

6

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Abstract

The importance of accessing high quality clinical samples for translational research is now firmly recognised. Traditionally these samples were collected and curated by individuals with an interest in a particular disease type. In recent years the idea of centralising and storing tissue collections in the form of tissue banks or biobanks has developed. As a result a number of biobanks have been established in many different countries. These can be either single centres or multi centre collaborations, often in the form of a federated network. This chapter outlines the development of breast tissue banking in a global context and discusses some of the challenges that lie ahead for the field, in particular how to meet the growing needs of researchers, how to make the best use of donated samples and how to increase the visibility of samples residing in biobanks to researchers.

Keywords

Biobanks • Breast cancer • Mammary gland • Tissue banking • Transitional research

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Abbreviations

ABCTB	Australian Breast Cancer Campaign Tissue Bank
BCNTB	Breast Cancer Now Tissue Bank
ER	Estrogen receptor
HER2	Human epidermal growth factor receptor 2
KTB	The Susan G Komen for the Cure Tissue Bank
PR	Progesterone receptor
TNBC	Triple negative breast cancer

6.1 Introduction

While the outcome for breast cancer patients has improved significantly over the past few decades, only three clinically-validated biomarkers are available which predict breast cancer response to endocrine (ER and PR) or biological therapies (HER2; reviewed in [1]). Moreover clinicians responsible for treating breast cancer patients still cannot readily predict which patients will develop resistance to treatments. In a series of two gap analysis documents, commissioned by the UK charity Breast Cancer Now, clinical and scientific breast cancer experts recognised that improved access to a source of carefully collected well-annotated human breast tissues, accompanied by matching clinical data can help solve these issues [2, 3].

Traditionally collections of tissue samples were instigated and curated by individuals with an interest in a particular disease type, often existing as 'private' collections. However there has been a gradual shift towards localising these within biobanks. As a result a number of generic and tissue-specific biobanks have developed. Many research institutions maintain such banks for research purposes however these tend to be independently run, often with their own cataloguing systems and databases, meaning there is limited interoperability. This can present challenges to breast cancer researchers seeking to identify sufficient tissue for research, as exemplified in the study by Curtis et al. who had to approach four different generic biobanks in order to accrue 2000 samples which they used to show that at least ten different subtypes of breast cancer existed [4].

A specialist breast tissue bank is attractive to researchers. Not only can this facilitate the process of sample accrual but it also eliminates the additional work needed by researchers when they are required to obtain breast tissue samples from multiple sources/locations. An added benefit is that samples from specialist banks are often collected uniformly according to standard operating procedures. Introducing standardisation can provide researchers with confidence in the results they obtain in subsequent downstream analysis. Well annotated follow-up data from patients is usually also available, which is essential in driving translational research. Furthermore, as breast cancer is complex and heterogeneous, rarer subtypes may not be well represented in generic biobanks. Tissue-specific biobanks can more readily accumulate these, particularly if they are part of a collaborating network. Triple negative breast cancer (TNBC), exemplified by its lack of expression of ER, PR and HER2, hence unsuited to current therapies which target these molecules, is a good example. TNBC is generally quite aggressive, often with poor clinical outcome. As a result there is considerable interest from researchers in understanding the biology of TNBC with a view to developing targeted treatments; currently these are limited to chemo- and radiotherapy, which have unpleasant side effects. However, TNBC only accounts for about 15 % of all breast cancers so it can take time to collect sufficiently large numbers needed to be able to perform a study with adequate statistical power. Specialist breast cancer biobanks, especially those which are multi-centre can speed up the process of sample accrual.

6.2 Breast Cancer Tissue Banks

A number of specialist breast cancer biobanks have developed in Australia, North and South America, Europe and Asia (Table 6.1). These operate either as single site banks or as hub and

Table 6.1 Specialist breast tissue banks around the world

Name	Location	Funding source	Ref.	URL (if applicable)
Argentinian Breast Tissue Bank	Argentina	Smith Grant Breast Center Bayloy College of Medicine	[5]	http://www.biobanco.com.ar/
Australian Breast Tissue Bank	Australia	National Health and Medical Research Council of Australia; National Breast Cancer Foundation; The Cancer Institute NSW	[6, 7]	http://www.abctb.org.au/abctbNew2/default.aspx
Breast Cancer Now Tissue Bank	UK	Breast Cancer Now	[8, 9]	http://bcctb.org/about-tissue-bank.php
Breast Tumor Bank	Romania	Not specified		None
Iranian Breast Cancer Biobank	Iran	Iranian Molecular Medicine Network	[10]	None
NCIC-Manitoba Breast Tumor Bank	Canada	Canadian Cancer Society	[11]	http://www.umanitoba.ca/institutes/manitoba_institute_cell_biology/MBTB/Index4.htm
PATH Biobank	Germany	Not specified	[12]	http://path-biobank.org/index.php/en/
St James's Hospital Biobank	Ireland	Vodafone Ireland Foundation	[13]	http://biobankireland.com/at-st-jamess
The Susan G Komen for the Cure Tissue Bank	USA	Susan G Komen for The Cure	[14, 15]	http://komentissuebank.iu.edu/

spoke consortiums. Following appropriate ethical/IRB approval most, if not all, collect fresh frozen material, formalin-fixed paraffin embedded samples and various blood derivative including serum and plasma. Tissue microarrays are sometimes available. Some also provide primary cell cultures, providing scientists with more clinically relevant alternatives for research to those offered by traditional breast cancer cell lines. The longest serving breast tissue bank is the NCIC-Manitoba Breast Tumor Bank, developed 20 years ago as a national repository to support breast cancer research in Canada [11]. A similar resource, The Australian Breast Cancer Tissue Bank (ABCTB) also exists [6, 7] and a Breast Biobank is being established in Argentina at the Breast Center in Buenos Aires [5]. In Europe there is a Breast Tumour Bank in Romania [16], the Patient's Tumour Bank of Hope (PATH) biobank, founded by breast cancer survivors, exists in Germany [12] and in Ireland the St James's Hospital Biobank was established to develop a high-quality breast tissue bio resource, integrated into the breast cancer clinical care pathway [13]. In the UK the need for a specialised breast biobank was recognised following a review by around 50 of the UK breast cancer experts of the barriers faced by scientists in translating promising laboratory research to the clinic. One of the key issues identified was lack of access to high quality breast tissue samples with appropriate clinical annotations [1]. As a result the Breast Cancer Now Tissue Bank (BCNTB) was established in 2010 as a coalition of four of the leading UK centres in breast cancer research providing a central access point to apply for breast tissues, biofluids and primary epithelial and stromal cell cultures derived from tumour and normal material [8, 9]. The BCNTB also developed a bioinformatics portal which allows researchers to mine existing breast cancer-omics data. To our knowledge the Iranian Breast Cancer Biobank is the only specialist breast cancer biobank in Asia. This commenced operations in 2005 and complements existing breast cancer biobanks in that it offers researchers the opportunity to obtain samples from patients from less common ethnic backgrounds [10].

6.3 Normal Breast Tissue

While the focus of translational research is often on cancer samples one should not overlook the importance of studying normal tissue as this may provide early clues as to what goes wrong during carcinogenesis; we still do not fully understand what causes breast cancer or how to prevent it. Normal tissues are sometimes available from breast cancer biobanks however this is often tissue adjacent to a breast lesion which may be subject to field effects of the neighbouring carcinoma, therefore not truly 'normal'. Alterations in gene expression of normal tissue adjacent to breast tumours have been reported [17] however subsequent work by the same group showed that histologically normal epithelium from breast cancer patients and from cancer-free prophylactic mastectomy patients shared a similar genetic profile [18]. It is also well recognised that the quantity of glandular tissue decreases with age and as breast cancer predominantly affects post-menopausal women the tissue available is often very fatty and non-glandular, as illustrated in Fig. 6.1. Breast tissue from the unaffected breast may also be available through an operation for symmetry. More commonly researchers turn to reduction mammoplasty material as a source of normal breast tissue. This tissue is left over from cosmetic procedures and is often plentiful. However, use of these tissues as controls has been found to be inadequate by researchers as histological comparison of truly normal breast tissue with that from reduction mammoplasties and benign breast disease showed that both of these cohorts displayed common pathological changes in the tissue [19–21]. These results cast doubt on the use of reduction mammoplasty material as a 'normal' control.

To our knowledge, the only biobank that collects truly normal breast tissue is the The Susan G Komen for the Cure Tissue Bank (KTB) at Indiana University Simon Cancer Centre in the USA [14]. This relies on voluntary donation of breast tissue and blood samples by healthy women with no history of breast disease and is open to all ages from 18 upwards. Tissue collections are scheduled over a weekend, often

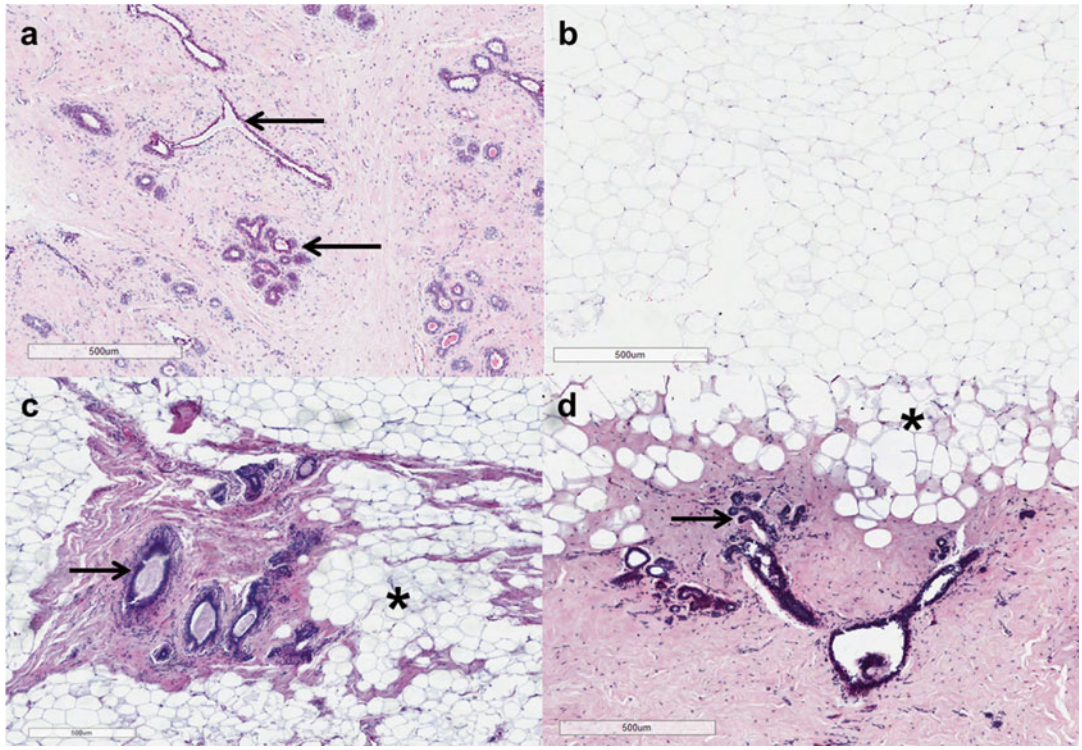

Fig. 6.1 H&E images showing examples of the histological appearance of normal breast tissue obtained from different donors. Normal tissue surrounding a breast tumour donated by a 33 year old patient is shown in (**a**). This shows typical histology associated with normal mammary gland with extensive ducts and lobules (*arrows*). This contrasts with (**b**) which shows normal tissue surrounding a breast tumour donated by a 68 year old patient. This section contained adipose tissue only. In (**c**) normal tissue from a pre-menopausal case undergoing risk reducing surgery had few ducts or lobules (*arrow*), but copious adipose tissue (**d**; *asterisks*). This is also reflected in (**d**), a reduction mammoplasty case from a 48 year old. All samples represent 4 μm formalin-fixed paraffin-embedded sections. Scale bars are shown on each image (500 μm)

designed to coincide with major sporting events e.g., Super Bowl with several hundred women turning up to volunteer [15] (V Speirs, personal experience). Using tissue obtained from KTB, Radovich et al. [22] concluded that breast tissue from healthy volunteers' acts as a superior normal breast tissue control. A complementary paper defined the transcriptome of normal healthy pre-menopausal breast tissue obtained from KTB using next generation sequencing [23]. This will provide a useful reference point for future comparison with breast cancer. Researchers do need to keep in mind that tissue from pre-menopausal women is subject to cyclical hormonal influences which may impact on some types of studies. However this information is recorded by KTB and is available if required. KTB has also generated normal primary mammary epithelial and stromal cells, showing significant plasticity of epithelial cells [24]. Along with the primary cell cultures of luminal, myoepithelial and fibroblast cells available from BCNTB [25], these offer unique tools for the in vitro study of breast biology.

Launched in 2008, the Love Army of Women [26] takes a different approach from a traditional biobank. Instead of collecting tissues and storing these for future use the Army of Women allows scientists to collect what they need when they need it. Here, potential study volunteers are encouraged to sign up online. Scientists then apply to the Army of Women for the opportunity to recruit volunteers for their research. Every study is reviewed for scientific merit, safety, and

ethical considerations. Following ethical/IRB approval, approved studies are posted on line and volunteers are additionally notified by email, allowing them to self-select potential studies they may wish to participate in. Study requirements range from information gathering through completion of online questionnaires, to physically collecting biological samples including blood, urine, saliva, breast fluid and tissue. The latter can be collected by the researchers themselves (provided they are capable of doing this), or via a specified Army of Women Center in the US. In a recent Army of Women study, women provided baseline (fasting) blood samples followed by samples taken at various time points after grapefruit intake to test the effect of this on endogenous serum estrogen levels in postmenopausal women [27]. A study currently listed on the Love Army of Women website and seeking recruits is "The Milk Study". This proposes to examine breast milk samples from lactating women who are scheduled for a breast biopsy. The idea is that analysis of promoter hyper-methylation in epithelial cells shed from ducts and found in expressed milk may provide an assessment of breast-cancer-risk. This is an interesting concept; many human milk banks exist, storing milk donated by nursing mothers [28] hence if the hypothesis proposed in "The Milk Study" is proved and appropriate ethical legislation can be overcome, milk banks could be used in future to evaluate breast cancer risk.

6.4 Challenges for Biobanks

While access to primary breast tissue was still recognised as a continuing need by the breast cancer research community, a subsequent gap analysis commissioned by Breast Cancer Now, this time involving over 100 breast cancer experts and overseen by an international steering group, identified an increasing need for longitudinal samples and, in particular, metastatic material [3]. The latter can be difficult to obtain as common metastatic sites site for breast cancer such as bone and brain are not very accessible. This presents challenges for biobanks and emphasises the need for cross disciplinary working to ensure samples being collected today meet the needs of researchers in the future. It makes sense for biobanks to work together to pool these rare resources. Patient advocacy groups can play a role to help make this happen (see advocacy chapter).

Biobanks have a responsibility to donors to ensure that the best possible use of made of donated materials [9]. For nucleic acid work, improvements in this technology have led to the development of dual extraction kits whereby DNA and RNA can now be extracted from the same piece of tissue, offering savings in cost and time while at the same time preserving tissues [29]. Other kits even offer simultaneous extraction of RNA, DNA, and protein. These could be an advantage for breast cancer biobanks; given the recognised heterogeneity of breast tissue it is possible that different aliquots of stored tissue may vary in phenotype. Furthermore, the impact of mammographic screening means that breast lesions are getting smaller; whilst good news for patients, smaller amounts of material present challenges to researchers involved in tissue-based studies, making multi-extraction kits more attractive. Indeed successful simultaneous extraction of RNA, DNA and protein using Allprotect® from normal and cancerous breast tissue has been reported [13]. Nevertheless this type of approach is only suitable if sufficient *high quality* materials are extracted, so this remains fit for purpose for subsequent downstream applications [30]. This is particularly important for some of the newer sequencing technologies which rely on input of high quality nucleic acids.

A further challenge biobanks face is the need to future proof samples such that stored samples can be used to take advantage of the latest developments in -omics technology. This is high priority. Regular horizon scanning by biobank custodians is recommended to keep abreast of the latest research developments and ensure collected samples remain fit for purpose. It is reassuring to note that archival serum collected from breast cancer patients and stored for 30 years were suitable for modern-day proteomic analysis, giving confidence that if correctly stored, such samples remain viable [31]. Similarly the

value of using archival breast tissue blocks stored for up to four decades has been demonstrated in biomarker studies [32]. As older samples will often have rich follow up data these could prove invaluable in biomarker discovery and in helping understanding breast cancer biology.

Finally biobanks have a responsibility to ensure work is not duplicated. To help eliminate this the BCNTB implemented a data return policy, meaning that all data generated from samples, either positive or negative, is returned to the bank within 2 years of study completion [9]. Other tissue banks are starting to follow suit e.g. the ABCTB also employs a data return policy [7].

6.5 The Economics of Biobanking

Biobanking is an expensive commodity. While biobanks often employ cost recovery mechanisms, to recoup staff, storage and consumable costs associated with sample procurement and storage, it is unlikely that full economic costs will be recouped. Additional support will be required from a variety of stakeholders, including academic institutions, government and charitable agencies. It is notable that the majority of the breast tissue banks highlighted in Table 6.1 currently rely on support from predominantly charitable sources and not government organisations.

A key responsibility for biobanks is to ensure that their collections are used and not simply stored. Regular review of current and future requirements of the breast cancer research community is recommended to help alleviate this – as exemplified in Breast Cancer Now's gap analyses [2, 3]. Nevertheless, concern about sample underuse was recently expressed through a survey of biobank managers in the US [33] and also via the BioBanking and Molecular Resource Infrastructure (BBMRI) network [34]. BBMRI [35] was established as a means of increasing visibility and facilitating access to bioresources collected across Europe [36]. As well as this pan-Europe initiative, many counties are now developing registers in order to increase the visibility of biobanks. Many of these have websites through which researchers can view and select suitable samples. Specialist breast tissue banks could work together by promoting complementary banks through links to each other on their websites. Taking this a step further would be for breast tissue banks to form strategic alliances; this is already happening in Ireland. However as exemplified by some of the difficulties faced during the first phase of BBMRI, developing a unified breast tissue banking network across different countries is not without challenge, with a number of ethical, legal, regulatory and societal barriers to be overcome.

As recently highlighted [37], the pharmaceutical industry is one of the biggest potential users of biobank samples, yet access by pharma to these samples is often difficult. Biobanks need to be aware of this and adapt their access policies accordingly to facilitate access by pharma such that samples are available for use. On a different note, a recent welcome development is the launch of a new journal the Open Journal of Bioresources in July 2014 [38], aimed at helping researchers discover specific bioresources through publication of short, peer-reviewed articles highlighting such resources. It is notable that a breast tissue bank, the ABCTB featured in the first issue of this new journal [7].

6.6 Summary

While significant progress has been made in understanding the biology of breast cancer over the last few decades, the heterogeneous nature of this disease means there are still deficiencies in some areas. With biobanking now firmly embedded as a discipline in its own right in translational breast cancer research, the necessary biological samples and corresponding data needed to fill these gaps area available. Going forward, these samples will aid researchers in the development of potential new drugs or diagnostic assays for future use in the clinic to help breast cancer patients. Researchers also need a better understanding of the processes involved in the very early stages of breast carcinogenesis. As such there is likely to be increased call on normal

breast tissue samples and it would be an interesting exercise to evaluate if the KTB model could be replicated outside of the US, to increase the volume and diversity of normal breast tissue available across different ethnic groups.

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Biobanking of Cerebrospinal Fluid for Biomarker Analysis in Neurological Diseases

7

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Abstract

Cerebrospinal fluid (CSF) reflects pathophysiological aspects of neurological diseases, where neuroprotective strategies and biomarkers are urgently needed. Therefore, biobanking is very relevant for biomarker discovery and evaluation for these neurological diseases.

An important aspect of CSF biobanking is quality control, needed for e.g. consistent patient follow-up and the exchange of patient samples between research centers. Systematic studies to address effects of pre-analytical and storage variation on a broad range of CSF proteins are needed and initiated.

Important features of CSF biobanking are intensive collaboration in international networks and the tight application of standardized protocols. The current adoption of standardized protocols for CSF and blood collection and for biobanking of these samples, as presented in this chapter, enables biomarker studies in large cohorts of patients and controls.

In conclusion, biomarker research in neurodegenerative diseases has entered a new era due to the collaborative and multicenter efforts of many groups. The streamlining of biobanking procedures, including sample collection, quality control, and the selection of optimal control groups for investigating biomarkers is an important improvement to perform high quality biomarker studies.

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Biobank • Cerebrospinal fluid • Neurology • Biomarkers • Quality control

7.1 Introduction: Relevance of CSF Analysis

Cerebrospinal fluid (CSF) is a very precious fluid of extremely high value for biomarker discovery and analysis in neurological diseases. Due to its specific location and close interaction with the brain parenchyma, many brain-specific molecules, such as proteins and metabolites are present in the CSF and reflect brain specific and pathophysiologic processes. The detection and quantification of these molecular constituents are used in clinical routine for various neurological diseases, including acute and chronic inflammatory neurological diseases, such as bacterial meningitis and multiple sclerosis (MS) [3–5]. In addition, the CSF biomarkers β-amyloid (1-42/1-40), total tau protein and phosphorylated tau protein are increasingly used to discriminate Alzheimer's disease (AD) patients from controls and non-AD dementia patients, and are integrated in research criteria for the definition of AD [6, 7]. There is a strong need however, to identify additional novel biomarkers for these and other neurological diseases that can serve as objective and cost-effective tools to support early clinical diagnosis, to predict prognosis, to monitor disease progression and to facilitate (neuroprotective) treatment development. Moreover, since a brain biopsy or obtaining ventricular or interstitial CSF is very invasive, the analysis of molecular constituents of the lumbar CSF provides a more practically feasible alternative. CSF molecules can reflect disease-specific dynamic changes due to brain pathology in patients and can as such provide leads to unravel ongoing pathological processes as well as for therapy development.

CSF is obtained by lumbar puncture, which is considered as relatively invasive compared with blood sampling. The most common risks or complications are headache and lower back pain. The prevalence of typical post-puncture headache is generally low (<5 %), especially if non-traumatic needles are used and especially in the elderly [8–10]. The procedure can be performed in the outpatient setting almost as a routine procedure: the patient can leave the clinic immediately after the procedure without problems. Nevertheless, the procedure is mainly performed for clinical indications and rarely for research purposes because of its invasive nature and consequently there are only few large biobanks. There is therefore a strong impetus to the CSF biomarker field to collaborate in large consortia to gain access to large cohorts. To that end, standardized sampling and storage procedures have to be in place. Standard operating procedures for CSF sampling have already been published [11]. In this review, we emphasize the importance of CSF biobanking, and discuss the state of the art knowledge on pre-analytical factors in CSF and current consensus protocols for CSF biobanking. Lastly, we address the value and challenges of long-term storage of CSF and initiatives to apply quality control in CSF biobanking.

7.1.1 What Is CSF?

The physiological function of CSF includes: (a) carrying and cushioning the brain to reduce pressure to the neurons residing in the lowest anatomical structures of the brain, (b) protecting brain tissue against shocks and contact to the scull bone and intracranial pressure differences due to changes in blood flow, and (c) serving as a transport medium for hormones, nutrients, and waste disposal [12]. CSF is mainly produced in

the choroid plexus (66 %), at the subarachnoid blood-CSF barrier structures, and from interstitial fluid drainage [13]. The classical idea of CSF flow, streaming through the distinct brain anatomic spaces and being mostly reabsorbed via the arachnoid spaces, is currently challenged since several studies suggest a more complex, not unidirectional, circulation of CSF [for review see [14]]. The total volume of CSF increases during development and aging, being 140 ml in young adults and 300 ml in the elderly. This means that at a constant production rate, total turnover decreases from 4.5 times a day in young adults to three times a day in the elderly.

7.1.2 What Is Unique for CSF Biobanking?

A biobank naturally contains clinical information linked to the biological samples. This information in fact determines the actual value of the samples, since well characterized patients and phenotypes with many clinical data are essential for the interpretation of biomarkers. In the field of neurological disease this means results of laboratory investigations, such as routine CSF and blood values, extensive clinical (long-term follow-up) data: data on clinical, cognitive and functional status, and often also imaging data. A unique aspect of CSF biobanks is the rare availability of samples from healthy controls. As mentioned previously, the collection process is considered invasive. Thus, ethical committees frequently raise concerns of performing the procedure on healthy volunteers and facilities or clinical researchers face high insurance costs to cover possible complications in this population. The field has therefore turned to alternative control groups in biomarker studies, such as disease controls or symptomatic controls. Since there was a heterogeneity in use and definition of such control groups, the BioMS-eu consortium has recently developed consensus guidelines for the definition and application of control groups in CSF biomarker studies [15].

7.2 Effect of Pre-analytical Factors on CSF Biomarkers During Biobanking

Pre-analytical variation can be an important source of variation in cerebrospinal fluid (CSF) biomarker analysis. In general, pre-analytical errors are known to account for 60 % of total laboratory errors [16], and are relevant for both diagnostic and research settings. The term 'pre-analytical variation' is used to indicate variation in many aspects of the total biomarker analysis process, ranging from patient related factors, variation in the CSF collection process to variation in assay performance (Fig. 7.1).

Compared to blood, CSF has a very different cellular and biochemical composition (e.g. 200 times lower protein concentration and lower protease activity) [17, 18], which likely leads to different protein stability compared to blood and thus other influence of pre-analytical variation is expected. Most CSF proteins originate from blood (85 %) via passive diffusion across the blood-CSF barrier [19, 20], and thus blood contamination can affect the CSF biomarker values of biobanked samples.

We here focus on pre-analytical factors relevant for CSF biobanking. We will expand largely on laboratory processing, to focus on CSF-specific effects and to carefully introduce the international consensus guidelines for CSF collection and biobanking. There are not many reports available in the literature addressing the effects of these issues specifically for CSF biomarkers. Nevertheless, the interest in the subject is increasing due to several international initiatives to improve the (diagnostic) analysis of CSF biomarkers and long-term biobanking [1, 11, 15, 21–23].

To generate recommendations for pre-analytical procedures, a distinction can be made between analysis of a specific biomarker in the clinical diagnostic setting and biobanking for research purposes, where samples are usually stored to identify novel biomarkers with unknown pre-analytical specifics. To illustrate this, adding

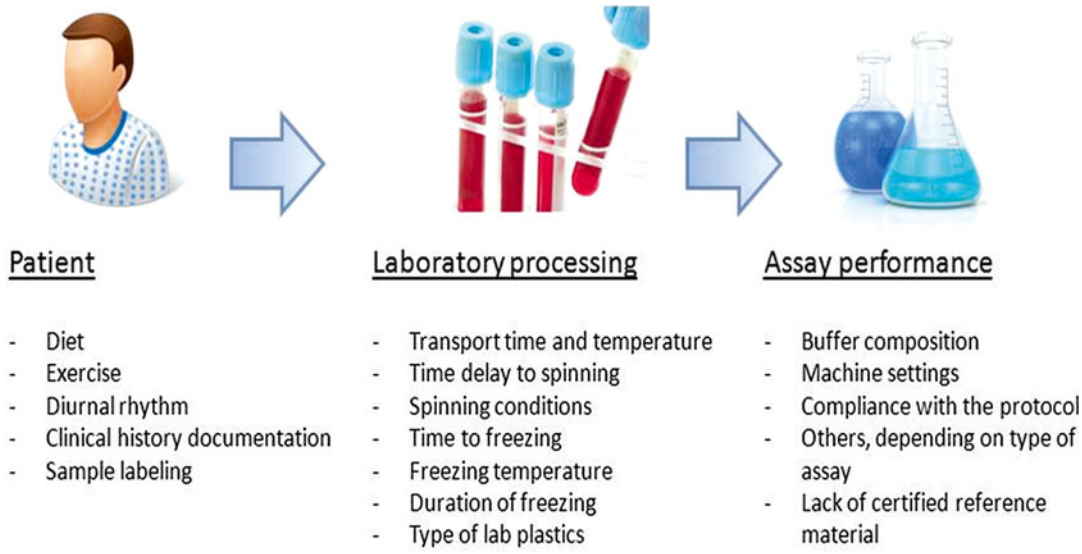

Fig. 7.1 Flow chart of pre-analytical steps in CSF analysis and common variability factors influencing outcome measures

Tween-20 to a CSF sample prevents amyloid beta (Abeta)(1-42) peptides from absorbing to several lab plastic surfaces [24]. Adding Tween-20 will decrease pre-analytical variation in these samples when they are collected for amyloid determination only. On the other hand, they will be unserviceable to many other purposes, e.g. studies targeting potential new biomarkers, because Tween-20 might interact with CSF components or the assays. In biobanking practice, we therefore advice to not add any substance to CSF samples, in order to avoid possible adverse effects.

Another major difference between routine analysis and biobanking for research purposes is the storage duration, which can be as short as one hour for routine tests which are often automated, including cell counts, protein concentration and IgG index, up to 1 month for special tests as oligoclonal bands, IgG index in some settings, and Alzheimer biomarker analysis by ELISA. The value of a biobank in the research setting, in contrast, increases upon long-term follow-up of patients and when cohort sizes are allowed to increase. Thus, storage of material in biobanks will be rather multiple years than months. Therefore, protocols for long-term biobanking purposes are more stringent and in particular require storage at −80 °C.

One approach to reduce variation due to patient issues and laboratory processing is to standardize CSF collection protocols as much as possible, which has been addressed by several consortia in recent years. The current protocols are in the majority based on the initial consensus guideline of the BioMS-consortium [11], that felt that the first step to improve the quality of biomarker studies and minimize variation between studies was to standardize the collection procedures. The most updated version of the protocol is presented in Table 7.1, which includes a standardized protocol for blood collection as well. The idea behind the protocols is to standardize every aspect during the CSF process to minimize variation, even if the rationale for specific decisions cannot as yet be given due to lack of experimental data.

In the following paragraphs, we elaborate on patient related factors and processing factors from the framework of Fig. 7.1 as these are most pertinent to CSF biobanking.

Table 7.1 Collection protocol for CSF and blood pairs for biobanking

Item no	Procedure	Ideal situation for CSF	Blood
A: Collection procedures			
1	Time of day of withdrawal and storage:	Record date and time of collection.	Same as for CSF
2	Preferred volume:	At least 12 ml. First 1–2 ml for routine CSF assessment. Last 10 ml for biobanking. Record volume taken and fraction used for biobanking, if applicable.	10 ml EDTA-plasma, 10 ml serum
3	Location:	Intervertebral space L3-L5(S1)	Venipuncture
4	If blood contamination occurred:	Do not process further. Criteria for blood contamination: more than 500 red blood cells/μL. Record number of blood cells in diagnostic samples.	Na
5	Type of needle:	Atraumatic	Standard needles, e.g. 21–23 G
6	Type of collection tube:	Polypropylene tubes, screw cap, volume >10 ml.	For serum: no clotting activator or gel For EDTA-plasma: no protease inhibitors
7	Other body fluids that should be collected simultaneously:	Serum	Na
8	Other body fluids that should be collected simultaneously:	Plasma: EDTA (preferred over citrate).	Na
B. Processing for storage			
9	Storage temperature until freezing:	Room temperature before, during and after centrifugation.	Same as CSF
10	Centrifugation conditions:	2000 g (1800–2200), 10 min at room temperature.	2000 g (1800–2200), 10 min at room temperature.
11	Time delay between withdrawal, processing and freezing:	Between 30 and 60 min. Max 2 h. After centrifugation, samples should be aliquoted and frozen immediately, with a maximal delay of 2 h.	Between 30 and 60 min. Max 2 h. Less than one hour is optimal for proteomics discovery studies. Serum must clot minimal 30 min.
12	Type of tube for aliquoting:	Small polypropylene tubes (2 ml for routine diagnostics; 1 ml for biobanking) with screw caps. Record manufacturer.	As CSF
13	Aliquoting:	A minimum of two aliquots is recommended. The advised research sample volume of 10 ml should be enough for >10 aliquots.	As CSF

(continued)

Table 7.1 (continued)

Item no	Procedure	Ideal situation for CSF	Blood
14	Volume of aliquots:	Minimum 0.1 ml. Depending on total volume of tube: 0.2, 0.5 and 1 ml. Preferably, the tubes are filled up to 75 % of the volume.	As CSF
15	Coding:	Unique codes. Freezing-proof labels. Ideally barcodes to facilitate searching, to aid in blinding the analysis and to protect the privacy of patients.	As CSF
16	Freezing temperature:	–80 °C	As CSF

7.2.1 Pre-analytical Variation Due to Patient-Related Factors

Patient misidentification or incorrect requesting are major sources of variation in laboratory settings and are not specific for CSF biobanking. These can be improved e.g. by the introduction of electronic patient records and linkage of these systems to research databases. The future of sample labeling in biobanking for research presumably lies in 2D barcoding, which will serve automated sample retrieving and picking, will further reduce mistakes and facilitates research using pseudonymized samples.

Item 1 of Table 7.1: Time of the Day of Withdrawal and Storage Patient-related pre-analytical factors can influence biomarker outcomes (Fig. 7.1). Such factors include effects of fasting, smoking, alcohol use, caffeine intake and exercise. It is conceivable that dietary factors would primarily affect specific blood markers rather than CSF biomarkers. However, fluctuations in fatty acid amide concentrations were measured in CSF of rats between day and night, due to differences in food intake [25]. Experimental evidence in humans is lacking and where compounds such as alcohol or smoking have effects on neurons [26, 27], they may also have an effect on CSF. There are few descriptions of CSF biomarkers being affected by these lifestyle factors. If there are effects of smoking or drinking on CSF biomarker concentrations, it will be hard to define if these are trait markers or should be seen as direct pre-analytical confounders. For all these factors, documentation is important for long-term biobanking and future research.

Studying the effect of circadian rhythm (and fasting) of specific proteins in CSF is not easy to perform, as repeated sampling within a person needs to be performed. Recent studies reported minor fluctuations in CSF protein levels due to diurnal rhythm, however, these hardly exceeded assay variation [28–30]. Another difficulty in standardization was that individual peaks in biomarker fluctuations made it hard to generalise the findings for the whole group [2]. Since there is no evidence that standardization of time of withdrawal will eliminate pre-analytical variation for an individual, this is not recommended for CSF biobanking. Since effects of diurnal fluctuation cannot be excluded for every biomarker, recording time of withdrawal is important for CSF samples in biobanks.

7.2.2 Pre-analytical Variation Due to Laboratory Processing

In this paragraph, we discuss the guidelines for CSF processing and biobanking as presented in Table 7.1, supported by experimental evidence when available.

Pre-analytical Variation Due to Laboratory Processing The CSF volume taken can influence the concentration of biomarkers. If a small volume is taken, the CSF will reflect the composition of the lumbar dural sack, whereas large volumes may reflect the rostral spinal or even ventricular CSF. Most molecules and cell numbers are not

equally distributed throughout the spinal fluid, and it is known that an increasing gradient in protein concentration from ventricular to lumbar CSF exists [19, 31]. Therefore, if biomarker levels in a sample from a puncture of 2 ml are compared to that in a puncture of 15 ml, this can lead to erroneous comparisons for specific proteins.

In a recent study though, CSF protein concentrations of the neurospecific protein S100B and the neuron-specific enolase did not differ between ventricular and lumbar CSF in the same patients, though the leptomeningeal beta-trace and the blood derived albumin did [32]. A proteomic study comparing the first and the tenth ml from one CSF withdrawal revealed only one protein, apolipoprotein C1, to be significantly increased among 41 identified masses of which 11 were proteins were identified [33]. Probably, the production location of a protein is crucial for the presence of a ventriculo-lumbar gradient in CSF [34], however, this does not solve the uncertainty for presence of CSF gradients occurring for novel proteins. Therefore, we generally recommend to collect a standard volume of CSF for biobanking, and at least record the volume. The first 2 ml can be used for basic CSF analysis and the remainder of the sample should be pooled before aliquoting. If this is not possible, the fraction of each portion should be recorded. Of course, a larger volume of spinal fluid will facilitate the number of possible analyses. It is well established that the volume of collected CSF (up to 20 ml) does not correlate with the risk of post lumbar puncture headache [8, 35, 36].

Item 3 of Table 7.1: Location of Puncture: L3-L5 Usually, CSF will be taken from location L3-L5, and only rarely from other locations such as the cervical cisterns or from the lateral ventricles (e.g. in case of a ventricular drainage). Location of the puncture should be recorded since it matters for a few peptides [31, 32] and it might affect concentrations of not yet detected CSF proteins.

Item 4 of Table 7.1: Removal of Bloody CSF Samples A traumatic tap causing blood contamination of CSF occurs in about 14–20 % of standard lumbar punctures [37]. For markers that have high blood concentrations, such as coagulation factors, blood contamination can lead to false positive results, while no effects have been reported for proteins from neuronal origin as neurofilament light and heavy chains [38]. Vascular endothelial growth factor (VEGF) and neuron-specific enolase (NSE) are predominantly present in blood platelets and are CSF biomarker candidates for some neurological and neurodegenerative diseases. NSE levels linearly rise with increased hemolysis of both serum and CSF [39]. Also, the presence of cellular components in the CSF influences the levels of these proteins, the effect of which will be minimized by centrifugation. For example, VEGF levels remained relatively stable after spinning while in non-centrifuged samples VEGF could still be released by blood plateles present in the CSF [40]. Similarly, presence of blood proteins lead to suppressed MALDI-TOF proteomic patterns in CSF, which was highly reduced after removal of the blood cells by centrifugation of the sample prior to initial freezing and subsequent analysis [17, 41]. A recent study showed lists of CSF proteins affected and not-affected by blood contamination, where more than one-third of the 665 detected proteins appeared to be affected [42].

Recording of erythrocyte count is essential to select CSF samples appropriate for the intended measurements. Based on the proteomics studies, CSF samples with an erythrocyte count up to 500/μL can be included for biomarker studies, though lower percentage contamination is preferred to avoid effects on as yet unknown molecules. It is highly recommended though to spin samples to avoid contamination from blood cells; this will, however, not solve the problem with contamination by plasma proteins. The use of markers as quality indicators to indicate hemolysis of CSF (e.g. hemoglobin alpha and beta chains) is indicated [41], but thresholds remain to be defined.

Item 5 of Table 7.1: Use of Atraumatic Needles There is no evidence that the type of needle for lumbar puncture influences biomarker concentrations. However, atraumatic or small

gauge needles are best tolerated by patients, and are associated with a lower risk for post lumbar puncture headache [8].

Item 6 of Table 7.1: Use of Polypropylene Collection Tubes Proteins can stick to lab plastics, and due to its relative low protein content, these effects may be more pronounced for CSF. Moreover, many collection tubes release polymeric components [43], that can in some cases affect immunochemical and mass-spectrometric assays. The Abeta(1–42) peptide in particular proves sensitive to different tubes used [44–47], and it is thus conceivable that as yet undiscovered biomarkers suffer from similar effects. The use of polypropylene tubes, with their low protein binding potential, was proposed for collecting CSF. Actually, experimental evidence did not confirm the advantage of tubes purely composed of polypropylene [46], and comparing multiple polypropylene tubes did not give congruous results [44]. Thus, harmonization of the tubes proves a worthwhile effort to reduce variation and introducing one tube type with the lowest binding capacity for universal use still lies ahead. The European JPND 'BIOMARKAPD' consortium was initiated to standardize and harmonize the use of biomarkers for AD and Parkinson's disease. This consortium decided to use so far a Sarstedt polypropylene tube (cat nr: 62.610.018, 10 ml collection tube, round base; cat no. 62.554.502, 15 ml tube with conical base to use if a pellet is to be kept). An additional component causing variation is transferring CSF to new tubes: the more transfers the more protein concentrations are reduced, especially for sticky CSF peptides such as Abeta(1-42) [47].

Items 7 and 8 of Table 7.1: Withdrawal of Serum and Plasma Linked to the CSF Sample It is important to collect and biobank matched serum and/or plasma samples for evaluation of CSF biomarkers because the concentration of a marker in blood often influences that in CSF. Blood collection is moreover needed to calculate the IgG index for diagnostic purposes, and to define the intrathecal origin of a biomarker and thus its specificity for the CNS [3]. For novel biomarkers of neuronal origin, these may even be suitable markers to be measured in serum/plasma. Blood tests are ideal for analysis of biomarker concentrations over time for monitoring of disease progression or even population screening, where CSF is found too invasive for.

A standardized blood collection protocol is provided in Table 7.1.

Item 9 of Table 7.1: Storage at Room Temperature Until Spinning and Aliquoting For CSF, there are no data available yet that support a preference for leaving the samples at either room temperature or 4 °C until processing. A direct comparison was made between neurofilament protein levels stored at room temperature or 4 °C, and no difference in protein levels was found [38]. CSF enzymes, such as adenosine deaminase and acid sphingomyelinase, do not have a decreased enzymatic activity when stored for a maximum of 24 h at room temperature [48, 49]. Therefore, processing at room temperature for both serum/plasma and CSF, including during and after spinning, is suitable for most studies. However, in the specific case that RNA is to be sampled from CSF immune cells, the CSF should be spun immediately or stored at 4 °C until processing.

Item 10 of Table 7.1: Standardized Spinning Conditions We propose to adhere to a standardized spinning protocol of 400 g for 10 min at room temperature for CSF if cells are to be collected, otherwise between 1800 and 2200 g (10 min at room temperature). For serum we recommend to spin at 2000 g for 10 min at room temperature. Not many experimental reports on this topic exist and different speeds within the 1500–2500 g range may affect the serum or plasma yield rather than affecting biomarker concentrations. For plasma and serum, temperature of processing is known to be critical for specific biomarkers, such as TIMP-1, probably due to degranulation of platelets at room temperature [50].

Item 11 of Table 7.1: Standardization of Time Delay Between Withdrawal, Spinning, and Freezing Multiple proteomics studies proved

stability of CSF proteome for up to 2 h after withdrawal prior to further processing [17, 41], however one study showed changes in metabolites and amino acids after 30 min before centrifugation [51]. Stability was shown for neurofilament heavy and light chain proteins for up to 24-h time delay between lumbar puncture and spinning, independent of the temperature as indicated at item 9 [38]. A proton nuclear-magnetic-resonance study showed stability of myo-inositol, glucose, acetate, and alanine levels; a substantial decrease of citrate; and an increase in lactate, glutamine, creatine, and creatinine levels after a 72 h delay in processing of CSF at room temperature [52]. After spinning, a delay of 2 h before storage did not result in changes in CSF protein profiles [53, 54], whereas delayed storage of more than 6 h significantly changes peptide profiles [55].

CSF stability studies suggest that the effect of time delay before spinning is more prominent than the delay in storing samples after centrifugation [51, 54]. Zooming in on Alzheimer biomarker levels, changes in these biomarker levels were found after a 24 h- [56] or 2 days- delay [57] whereas studies testing delay between processing and storage report stability after a delay of several days [58, 59], or up to 14 days [60].

Thus, despite actual experimental data published on effects of delay either before centrifugation or between centrifugation and storage, results remain discrepant. It is clear though that stability of AD biomarkers and probably many other biomarkers can be guaranteed following the proposed guideline, which does not exceed a 4 h delay in total until storage in biobanks (2 h before and 2 h after spinning). New biomarkers should however always be tested on these aspects of stability.

Item 12 of Table 7.1: Use of Small Polypropylene Tubes for Aliquoting Due to the same rationale as for CSF withdrawal (item 6), we recommend that polypropylene tubes should be used for aliquoting. Furthermore, vials with screw caps should be used for a secure sealing and should be properly closed during handling for long-term biobanking. The proposed tube size is usually 2 ml for routine biomarkers and 1 ml for biobanking, however, since tubes should preferably be filled up to 75 % other tube volumes are used in practice too. The importance of preserving an as high aliquot volume to tube surface ratio as possible was underscored by a recent study on CSF levels of AD-associated Aβ42; small CSF aliquots resulted in loss of Aβ42 by adherence of the peptide to the test tube wall, which was a much smaller problem if the tube was properly filled [61].

Item 13 of Table 7.1: Aliquoting Freezing and thawing cycles can influence CSF biomarker concentrations, as was also shown for some specific blood biomarkers [62]. Studies show conflicting results for Alzheimer diagnostic CSF proteins after several freeze-thaw cycles. One study observed a significant loss of Abeta(1-42) after one freeze-thaw cycle [63], while others see no effects in Abeta(1-42) at all after up to three times freeze-thawing [59, 60], but rather a small decrease in Tau proteins after two to three cycles [60]. Another study found a 20 % decrease in Abeta(1-42) after minimum three thawing cycles [57]. The discrepancy in these results might lie in as yet unidentified other pre-analytical variables that differed between these studies.

Levels of several cytokines, vascular endothelial growth factor (VEGF), matrix metalloproteinase-2 (MMP-2), and neurofilament proteins were not affected by repeated freezing and thawing [38, 40, 64, 65].

Spectroscopy techniques show contradictory data too, e.g. no effects on CSF proteome profiles as determined by MALDI-MS have been observed after up to four cycles in a proteomics study [41]. On the other hand, a Raman spectroscopy study showed a decrease in peptides, also Abeta(1-42), after one freeze-thaw cycle [53].

Altogether, freeze-thaw cycles should be avoided in principle, as data addressing this topic are available for only a few molecules and the response to freeze-thaw cycles of new molecules is not known. Thus, splitting the samples in multiple small aliquots is recommended. As indicated at item 2, the total CSF sample must be pooled before aliquoting to avoid concentration gradients.

Item 14 of Table 7.1: Volumes of Aliquots of 0.2, 0.5, and 1 ml (Depending on Total Volume of Tube and Minimally 0.1 ml) Storage in small aliquots prevents CSF from degrading due to freeze-thawing. Tubes should preferably be filled up to 75 % to preserve concentration, since protein concentrations could be influenced by evaporation and absorbance of CSF components to the surface of the tube wall and bottom. The true influence of both factors is under current study, but initial data show that evaporation is not relevant during 2 years of storage in at least one type of tube [21]. Preferred tube sizes are indicated at item 12.

Item 15 of Table 7.1: Coding and Use of Freezing-Proof Labels As for all biobanks, samples need a unique pseudonymized coding which is coupled to a protected database providing e.g. clinical and imaging information or informed consent information. Barcoded sample tubes are preferred, since it enables automation, permits double-blinded research effortlessly and it protects the privacy of the patient. Labels should be suitable for prolonged storage at −80 °C.

Item 16 of Table 7.1: Freezing Temperature of −80 °C Most large proteins such as antibodies are stable at −20 °C for several months [66]. However, epitopes of smaller molecules may change due to oxidation resulting from pH changes during storage [67] and this could effect protein concentrations, which will also depend on the particular assay used for detection. A recent review evaluated the effect of storage temperatures on human biospecimens preserved in biobanks [68], but there is little published data on the effect of storage temperature on CSF constituents. Effect of storage of CSF at −20 and −80 °C on cystatin C was investigated by mass spectrometry. Cleavage of eight amino acids of this protein occurred in samples stored at −20 °C but not in samples stored at −80 °C [17, 41, 69, 70]. Apart from the cystatin C truncation, changes in the remainder of the low molecular weight polypeptide profile due to CSF sample storage at −20 °C for 3 months appeared to be minimal [17, 41]. Activity of secretory acid sphingomyelinase was increased when CSF samples were stored at −20 °C for 2 months compared to at −80 °C [49], indicating that storage at −20 °C is not sufficient for preservation of the true activity of this enzyme. Neuron-specific enolase was found to be instable when CSF was stored at −20 °C (27 % concentration decrease after 1 month) and better conserved at −80 °C (22 % concentration decrease after 9 months) [39]. Oligoclonal bands in CSF may be recovered after several years of storage at −20 °C indicating a high stability of immunoglobulins [66]. Short-time freezing of samples at −80 or −196 °C did not change the CSF peptide profile [55].

There might be a beneficial effect of quick freeze-drying CSF in liquid nitrogen prior to storage at −80 °C, since the protein conformation seems less effected when compared to slow freezing at −80 °C [53]. For many specific CSF markers it is not known how freeze-drying effects their conformation and binding capacity, and it is conceivable that conformational change may be the cause of observed effects of repeated freeze-thawing. Since freeze-drying in liquid nitrogen is an expensive technique and not available in all laboratories, it is not implemented in the protocols.

Taken together, we recommend that samples should be transferred to −80 °C as soon as possible to ensure long-term stability of biomarkers. For specific biomarkers already implemented in the diagnostic work-up, such as Abeta(1-42) and oligoclonal bands, short term storage at −20 °C will not be problematic.

7.2.3 Effects of Long-Term Storage of CSF

Long-term biobanking of human body fluids is of great importance for the discovery of novel biomarkers, but the effects of long-term storage on molecular constituents of CSF or blood are not known. There are few reports published on this, indicating stability of Abeta(1-42) and Tau proteins for at least 6 years [71]. Using an Arrhenius plot, these CSF biomarkers, and also neurofilament proteins, were predicted to be stable during storage at −80 °C [57], but real experimental evi-

dence on this subject is lacking. The effects of long-term storage on different CSF molecules and potential beneficial effects of additives need to be established. Structural changes occurring in molecules due to freezing (and thawing), and possible effects of evaporation suggest that there could indeed be changes in CSF measures after long-term storage. Hopefully, experimental data in the future will contribute to our understanding of processes during long-term storage on a molecular level since more and more samples are stored to be used for biomarker research.

7.2.4 Quality Control in CSF Biobanking

Many multicenter studies revealed that different biomarker outcome levels and even cut-off levels are used in different laboratories, even when using the same commercial kits [72–74]. A recent study took a close look on this, and found that intra- as well as interlaboratory variation led to a change in diagnosis in respectively 26 % and 12 % percent of the cases, after re-analysis based on the Aβ42 biomarker [75]. This again accentuates the impediment of joint large-cohort studies by pre-analytical variation and the need to find a broadly applicable approach to cope with it.

Another endeavor to solve pre-analytical variation in CSF, complementing standardization efforts, would be to use an independent quality marker for CSF. This should be an instable molecule whose instability relates to a specific pre-analytical step in the process, functioning thus as a sentinel molecule for decreased quality of the CSF sample and preferably predicting the potential influence on the outcome of the protein of interest. Presumably a panel of sentinel molecules is required, together covering each laboratory step causing pre-analytical variation, such as delayed storage, freeze-thaw cycles, duration of storage, temperature during transport, spinning and storage, since they might all affect quality in a different manner.

The International Society for Biological and Environmental Repositories (ISBER) Biospecimen Science working group recently reviewed the current state of the art of quality control of human biospecimens [76]. Promising reports were published recently revealing several enzymes in blood whose levels were changed upon variation in laboratory processing, reflecting the effect of specific pre-analytical steps [77, 78]. These studies need replication and optimization to be implemented in the biobanking processing protocol. In CSF, the identification of such quality markers maybe complicated by the fact that CSF proteins are less abundant and sometimes below detection threshold and consequently fewer assays are available for screening of such effects.

7.3 Outlook

Biomarker research in neurodegenerative diseases has entered a new era due to the collaborative and multicenter efforts of several networks. The streamlining of biobanking procedures, including quality control, and the selection of optimal control groups for investigating biomarkers is an important improvement to perform high quality biomarker studies. All these activities will ultimately lead to large cohorts of long-term biobanked specimens that can be used with confidence in collaborative multinational biomarker studies.

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Biobanking in the Twenty-First Century: Driving Population Metrics into Biobanking Quality

8

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Abstract

Biospecimens are the essential substrates for human biomarker research. Across the globe, biobanks have developed the facilities and mechanisms to collect, process, store and distribute those substrates to researchers. However, despite some notable successes, less than one hundred of the tens of thousands of purported biomarkers have been independently validated. We propose the need for a new paradigm in biobanking; simply pursuing larger numbers of participants, larger networks of biobanks and higher sample integrity will not, in itself, transform the success rate or efficiency of biomarker research. We propose that biobanks must embrace the intrinsic observational nature of biospecimens and furnish the recipients of biospecimens with the population metrics (descriptive statistics) that can facilitate the scientific rigor that is mandated in other areas of

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observational research. In addition, we discuss the value of population-based ascertainment and recruitment and the importance of the timing of biospecimen collections. Any assessment of biospecimen quality must go beyond the sample itself and consider both the patient/participant selection and the most appropriate and informative timing for specimen collection, particularly prior to any treatment intervention in diseased populations. The examples and rationales that we present are based largely on cancer-related collections because the feasibility of population metrics is greatly assisted by the comprehensive registries that are more common for cancer than other chronic diseases. Changing the biobanking paradigm from tacitly 'experimental' to explicitly 'observational' represents a profound but urgent methodological shift that will influence the establishment, management, reporting and impact of biobanks in the twenty-first century.

Keywords

Biospecimen • Biomarker research • Observational • Population-based • Epidemiology • Selection bias • Validation • Networking and integration • Biobanking quality • Cancer • Registries

8.1 Introduction

Research and healthcare-related biobanks have existed for over 60 years and are, as such, a twentieth century phenomenon. However, a three-fold increase in their number has occurred in the past decade [1]. This dramatic increase has been primarily fueled by the acknowledged necessity for biospecimens to support translational research and personalized medicine (the practice, or potential practice, of using predictive biomarkers, measured in the blood or tissues of patients, to select optimally effective therapies) [2–5]. Validated predictive biomarkers offer the potential for the selection of optimal treatments based on the likelihood of benefit, thereby avoiding unnecessary costs and ineffective therapies [6]. Personalized medicine is a particularly high priority in the treatment of cancer because conventional therapies are expensive, often highly toxic and cause long-term negative health impacts [7]. In addition, extensive cancer-related biospecimen collections have been established for prevention-focused research including the pursuit of non-invasive biomarkers for the early detection of cancer, assessment of cancer risk and estimation of biologically-effective doses for candidate risk factors [8, 9]. Consequently, cancer-focused collections comprised the largest proportion of biobanks in a recent survey [1]. In 2002, the National Cancer Institute (NCI) commissioned an internal and external review of biospecimen resources that led to the creation of the NCI Biorepository Coordinating Committee (BCC), the Office of Biorepositories and Biospecimen Research (OBBR) in 2005 and the Biospecimen Research Network (BRN) in 2007 [5]. One conclusion of this review, and subsequent attempts to use archived samples, was that the research utility of the samples (approximately 300 million and associated data stored in biobanks across the USA) was undermined by the variable quality and incomplete clinical annotation of these biospecimens [2].

The application of reliable biomarkers has the potential to transform health-care, particularly in

the prevention, early detection and treatment of cancer but very few have reached widespread clinical implementation despite enormous investments in their attempted identification, development and implementation [10, 11]. Indeed, the biomarker research literature is replete with examples of biomarker "discoveries" that have failed the test of independent replication and validation [12]. The very low proportion of biomarker associations that have been independently validated has typically been attributed to inadequacies in the original study designs and consequent high likelihood of chance or biased findings [13]. Bias and chance are fundamental considerations in observational research [12]; and must be carefully considered in the design and reporting of all biomarker studies and the activities of biobanks. Chance findings may be the product of measurement error but these can be mitigated by ensuring that an adequate sample size is proposed in the study design. However, even if the sample size is adequate, the risk of chance findings is particularly high in biomarker research where high-dimensional 'omics' data may be *over-fitted* [12, 14]. Over-fitting occurs when multivariate models are generated from high-dimensional data, and large numbers of independent variables are fitted to a small number of observations. When over-fitting occurs, there is a significant risk that the derived models describe random error rather than a true relationship. Independent validation of any model is essential since "over-fitted" models will fail subsequent independent validation. The likelihood of chance findings can be reduced by addressing sample size, measurement error and disciplined analysis. However, bias cannot be eliminated by simply increasing sample size or by statistical adjustment; it must be addressed in the study design and by careful interpretation [13].

Biospecimens for human research are invariably collected from free-living humans; even in the most tightly-controlled clinical trial settings, it is impossible to control every potential source of variation in a biospecimen. Therefore, by definition, human biospecimens represent observational data [15]. Experimental research is defined by creating a system in which all conditions are identical except for the single treatment or intervention being investigated. In contrast, observational research is defined by the measurement of characteristics across a system or population and the absence of any research-directed intervention or attempt, by the researcher, to modify behavior in any way. The distinction between experimental and observational studies has substantial implications for the process of biobanking, the conduct of biospecimen-related research and for biomarker identification and development. Failure to consider the requirements and address the limitations of observational data has been a contributory factor in the failure to translate seemingly promising initial studies into viable clinical tests [13]. Possibly the greatest opportunity for biobanks to enhance the quality of biomarker research in the twenty-first century is for biobanks to acknowledge the observational nature of human biospecimens explicitly and fully address the specific methodological issues and biases that they share with all observational research.

Randomized clinical trials (RCTs) are frequently referred to as experimental studies; however, elaborate methods, including randomization, blinding and reporting transparency, have been incorporated into clinical trials to emulate experimental approaches. In reality, these methods can mitigate, but never fully eliminate, the inescapable observational aspect of conducting clinical trials on free-living humans [16]. Several guidelines have been published in an effort to improve the reporting of clinical trials and observational studies. The Consolidated Standards of Reporting Trials (CONSORT) guidelines were developed specifically to improve the reporting of clinical trials but also, by implication, ensure that these elements were appropriately considered during the trial design and conduct phases. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement and guidelines [17] provide the equivalent advice for the conduct of observational studies. However, the inherent observational nature of human biospecimens and biomarker research is generally under-appreciated so these guidelines are not routinely applied by authors or requested

by journals. The Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria [18] have been adopted by some journals but specifically relate to prognostic marker studies and do not explicitly address the observational nature of biospecimen research. Given the lack of guidelines specific to the reporting of biospecimen-related research, the STROBE and STROBE–Molecular Epidemiology (STROBE-ME) [19] guidelines are currently the most relevant source of advice to enhance the reporting of biomarker research [20].

In 2001, the NCI-founded Early Detection Research Network (EDRN) proposed a five-phased approach for studies intended to identify biomarkers focused on the early detection of cancer [21, 22]. The EDRN originally endorsed the use of convenience samples in the early discovery phases (phases 1–2) as a pragmatic response to the understandable urgency to exploit the rapidly advancing technologies of the previous decade. Much of the research conducted over the past two decades has favored biomarker discovery over validation and accepted less stringent rules of evidence [23]. However, in more recent publications, the EDRN has changed its position on the use of convenience samples and advocates strongly for the use of "population – science principles in the design of biomarker discovery studies" and proposed the PRospective-specimen collection, retrospective-Blinded-Evaluation (PRoBE) guidelines [24].

Translational biomarker research is dependent upon the availability of large series of high-quality biologic samples and associated data [3]. However, the limitations of some existing biospecimen collections, for this purpose, have been demonstrated by the low success rate in replicating and externally validating the results of several high-profile biomarker "discoveries". Therefore, while the twentieth century represented the dawn of the human biobanking era, it is clear that biobanking in the twenty-first century must learn from the limitations of those pioneering initiatives to improve the quality of their resources and deliver on the promise of personalized medicine in routine care. If twenty-first century biobanks are to help realize the full potential of biomarker research, they must provide transparent reporting of biospecimen selection and deliver optimal substrates to facilitate robust and reproducible research.

8.2 Population Metrics to Improve Biomarker Research

There is an implicit assumption that the samples provided by biobanks are representative of the population, disease, or subset of disease that the researcher intends to investigate. However, many biobanks are based on opportunistic or convenience collections of biospecimens or are restricted by the strict inclusion criteria of clinical trials-related collections [1]. While clinical trials are designed to maximize internal validity, their external validity is often limited [25–27]: fewer than 5 % of cancer patients participate in clinical trials and trial participants tend to be younger, have better performance status and fewer co-morbid conditions compared to non-participants [27]. Consequently, the participant selection introduced through opportunistic or non population-based biospecimen collection can lead to over or under-representation of particular subgroups within a collection to the point that the collection of samples, on which future research is conducted, do not accurately reflect the disease burden or population at risk [28–30]. If the potential impact of participant selection is not considered in the downstream research conducted on those biospecimens, then that research may be compromised by selection bias. The significance of this potential selection bias may be exacerbated if samples are provided to experimental/laboratory-based researchers who are unaccustomed to considering the potential for such bias and the impact this might have on the interpretation and validity of subsequent results.

One of the defining features of a biobank is that the samples are associated with data [31]; those data can potentially be used to determine whether subjects (represented by their biologic samples) legitimately represent the population or disease entity under study and whether, or not,

any baseline inequalities exist between comparator groups for analyses. However, few biobanks routinely collect or distribute these data and laboratory-based researchers may be unaware of the potential value of these data and consequently, are unlikely to request them. If the inefficiencies that characterized much of the existing biomarker discovery research are to be reduced in future research, it is essential that biobanks equip themselves to provide appropriate clinical and/or population metrics (descriptive statistics) so that internal and external validity can be adequately assessed. To minimize the impact of potential selection bias, biobanks must strive to ensure that biospecimens are representative of the population at risk of the disease or the outcome of interest. These concepts are standard considerations in design of other observational research but they are rarely invoked until the latter stages of biomarker development research or by biobanks. Mandating the inclusion of population metrics into biospecimen collections and distributions would help researchers (biospecimen recipients) interpret findings appropriately and eliminate many of the weaknesses that currently undermine the discovery phase of biomarker research.

8.3 Implementing Population-Based Biospecimen Collection

Population-based ascertainment and recruitment provides the most broadly representative cohorts and the greatest likelihood that the participants represented will contain the relevant sub-groups that might be requested by future researchers for more focused/targeted investigations. Achieving population-based biospecimen collection requires the integration of three components: ascertainment (identifying potential participants), recruitment (facilitating their consent to participate in research) and evaluation (comparing the composition of those cohorts to the source population). Each of these components presents specific challenges. The challenges of participant/patient ascertainment have increased over the past two decades; in many countries, concerted efforts have been made to protect potentially sensitive personal information [32, 33]. Unfortunately, these well intentioned initiatives have created additional challenges for conducting population-based research [34]. Implementing population-based ascertainment and biospecimen collection, whether from non-diseased or diseased populations, must comply with the relevant data access regulations and is dependent on the public health and healthcare delivery system [35].

In addition to the challenges of accessing administrative data and databases to facilitate potential participant contact, administratively-fragmented and geographically-distributed healthcare and public health systems present substantial logistic hurdles to obtaining population-based cohorts. Approaches that rely on single geographic sites or healthcare providers are susceptible to systematic selection biases. For example, patients seen at a given site or by particular physicians may represent an extreme distribution of disease severity, age or socioeconomic status, all of which can potentially introduce biases into estimates of disease associations [29]. Therefore, the ideal biobank should apply strategies to identify, recruit and collect biospecimens from all eligible subjects or patients in order to minimize the impact of selection bias in their biospecimen inventory.

One approach to facilitate population-based cancer patient ascertainment for biospecimen collection is to establish a partnership with a comprehensive cancer registry. In North America, all central cancer registries are members of the North American Association of Central Cancer Registries (NAACCR) which promotes data standards and certification through independent annual review of data from member registries for their completeness, accuracy and timeliness [36]. If case ascertainment cannot be fully integrated with a comprehensive registry, relevant registries may still be used as a reference to which the population metrics of the biospecimens can be compared. These data can be obtained from cancer surveillance organizations like the Surveillance, Epidemiology, and End Results (SEER) program

in the USA and the European Network of Cancer Registries (ENCR) in Europe. Comprehensive registries are mandated to collect details of all cancer diagnoses in their jurisdictions and, in most cases, support relevant and appropriately approved research. However, partnering with a comprehensive registry merely provides a mechanism to identify relevant cases and reconcile ascertainment with incidence; the recruitment of participants and collection of biospecimens requires additional layers of complexity. Effective population-based recruitment of cancer patients typically requires multiple redundant systems for the rapid identification of patients of interest and sophisticated systems to track patient trajectories, consent, and biospecimen collections.

8.3.1 Advancing Biobanking in Alberta, Canada

The Alberta Cancer Research Biobank (ACRB) was established in 2006 and has been conducting population-based ascertainment and recruitment of breast cancer patients since 2010. In order to achieve our recruitment targets, we implemented seven distinct patient identification strategies within our healthcare system to facilitate the ascertainment and then recruitment of cancer patients prior to a treatment intervention (Table 8.1). Individually, each of the strategies, outlined in Table 8.1, captures a subset of cancer patients and, if used in isolation, could potentially introduce selection bias for downstream research. However, when combined into a multifaceted, comprehensive patient ascertainment strategy, the achievable response rates are high and represent a high proportion of the provincial cancer patient population for selected cancer types. However, even with this comprehensive approach, it is important to compare the disease and patient parameters between participating patients with those who decline participation, in order to assess any potentially skewed patient selection.

Identifying and recruiting participants diagnosed with specific diseases presents the challenge of the time-sensitivity for biospecimen collection before treatment. However, recruiting members of the healthy population to establish a population-based observational cohort presents different challenges. In 2000, the province of Alberta invested in a 5-year feasibility study to determine whether a cohort of adults could be established to study chronic disease etiology and to incorporate biospecimen collection for future biomarker studies. This feasibility study was positive and phase I of *Alberta's Tomorrow Project* subsequently enrolled approximately 31,000 Albertans by early 2008 using random digit dialing [38, 39]. Broad inclusion criteria were used: no prior history of cancer other than non-melanoma skin cancer, able to complete written questionnaires in English and planning to reside in Alberta for 1 year following recruitment. During phase I, participants completed mail-in questionnaires about health and lifestyle, physical activity and diet, and also consented to long-term follow-up and linkage with administrative health databases. In 2008, *Alberta's Tomorrow Project* joined British Columbia, Ontario, Quebec, and Atlantic Canada to form the *Canadian Partnership for Tomorrow Project*, a pan-Canadian platform comprising 300,000 adults aged 35–69 years who have consented to long-term follow-up (up to 50 years) and linkage with administrative health databases. In Alberta, the *Canadian Partnership for Tomorrow Project* protocol included collection of biospecimens, physical measures, and updated health and lifestyle information from re-consented phase I participants and newly recruited participants from across the province.

A common challenge in recruiting research participants, whether healthy or with specific diseases, is providing the opportunity to obtain informed consent and the collection of biospecimens, and other relevant measures. *Alberta's Tomorrow Project* established the appropriate conditions for these tasks by establishing a permanent study centre in Calgary, and deploying a fully-equipped mobile study team who traveled throughout the province. Together, this created the combined capacity to collect, process and store biospecimens from over 350 participants per week. Biospecimens collected at study centres included 50 mL of non-fasting blood and a

Table 8.1 Seven patient ascertainment and recruitment strategies to facilitate population-based biospecimen accrual and the potential selection biases associated with each individual approach

Identification method	Description	Potential selection biases
Cancer Registry[a]	Pathological evidence of a positive cancer diagnosis provided by a cancer registry.	Cancer registries may not capture 100 % of patient populations and/or may not identify patients for a biobank prior to treatment.
Direct Clinician Referral	Collaborations with key high-volume clinicians including surgeons, radiologists and oncologists actively consent patients for biobanking during their pre-treatment consultation	Not all clinicians are supportive or have the time and/or resources to support biobanking initiatives.
Surgical Booking Request	When a patient is diagnosed with a resectable cancer, a surgical booking request is generated to secure a surgery date and surgical suite.	Only includes patients scheduled for surgical treatment of their cancer.
Pre-Admission Clinic	The pre-admission clinic ensures that patients are prepared for a scheduled operation or procedure.	Often biased towards patients with significant co-morbidities and/or are considered at high risk of complications during a medical procedure.
Day Surgery Unit (DSU)	Patients are identified on the operating room slate and encountered in the DSU just prior to their surgery on the day of the operation.	Only includes patients treated for cancer with surgery/excision.
Pre-treatment Patient Education	Numerous programs are available to educate and inform patients prior to treatment.	Patient education sessions are not mandatory; only subsets of broader populations attend these sessions.
Nurse Navigator Referral	Oncology nurses are assigned to patients to help them navigate the continuum of cancer care. They may consent patients for biobanking and/or notify a biobank that a patient has entered their program [37].	Not all nurse navigators are supportive of biobanking and/or notify the biobank of patients entering their program.

[a]Additional ethical considerations involving the patient's awareness of diagnosis must be addressed prior to contacting a patient to obtain informed biobanking consent

spot sample of urine. Blood was fractionated into serum, plasma, buffy coat and red blood cells; multiple aliquots of each fraction are stored in 2D bar-coded tubes at −80 °C. The ambitious processing protocol resulted in the majority of blood samples being processed and transferred to −80 °C storage within two hours of collection. Participants were also measured (standing/sitting height, weight, body composition, grip strength, waist and hip circumferences, blood pressure, resting heart rate), and asked to complete a short questionnaire describing behaviors prior to donating blood and urine. Participants who were unwilling or unable to attend a study centre were given the option of providing a saliva sample (Oragene, DNA Genotek Inc., Ontario, Canada) by mail. Currently, *Alberta's Tomorrow Project* is in the process of extracting DNA from buffy coat samples and the saliva kits, and anticipates making samples available to *bona fide* researchers by Fall 2015. While every effort has been made to apply methods to maximize the representativeness of *Alberta's Tomorrow Project*, comparison with the Canadian Community Health Survey (Cycle 1.1) has been a useful approach to explore if and how the cohort in *Alberta's Tomorrow Project* differs from the provincial population [39]. Plans are in place to update these analyses for the newly enrolled participants.

8.4 Integrated Biobanking Models

Reigmans et al. [40] broadly classified biobanks into three types primarily based on the purpose of biospecimen collections (Fig. 8.1). Collections that were established to support population-based prospective cohorts were termed *population biobanks*. These biobanks typically collect non-invasive biospecimens (such as blood or exfoliated buccal cells) plus extensive lifestyle data from asymptomatic "healthy" cohort participants, often for subsequent nested-case control studies and with a focus on supporting etiologic and early detection research. *Alberta's Tomorrow Project* and the *Canadian Partnership for Tomorrow Project* are examples of population biobanks. *Disease-oriented biobanks for epidemiology* were considered as a distinct category; these biobanks often focus on exposure assessment in high-risk populations and collect significant epidemiologic data to accompany the biospecimens collected. The archetypal "tumour banks", typically associated with clinical institutions, were termed *disease–oriented general biobanks* and these predominantly focus on the collection of tumour tissue and, less commonly, blood and other specimen types [40]. Since these categories were proposed, they have been widely adopted and provide a helpful nomenclature to identify the key roles and contributions of particular biospecimen resources.

Although each of these biobank types support important aspects of the translational research continuum, considerable synergy can be achieved through integrating two or more of these approaches (Fig. 8.1). The benefits of combining

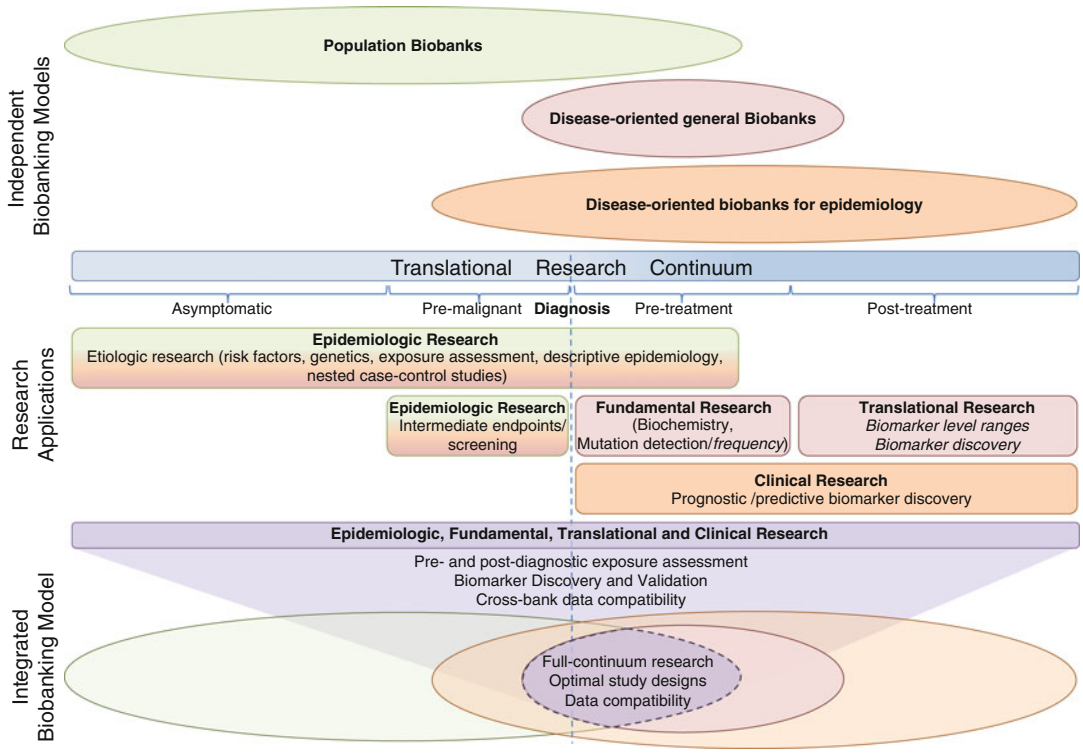

Fig. 8.1 Research and operational benefits of integrated biobanking models. Leveraging the expertise, facilities and personnel provides operational efficiencies. Robust epidemiologic oversight, data compatibility and uniformity of specimen handling support enhanced research capacity (labels in *Italics* indicate common application of specimen types that are potentially susceptible to biases)

biobanking activities are illustrated by considering the limitations of each approach in isolation. For example, *population biobanks* are necessarily a long-term investment. The typically low event rates require that a large population be enrolled at baseline and the accumulation of sufficient numbers of outcomes, to test associations with disease endpoints, can take many years or decades. In isolation, there is a risk that such cohorts and biospecimen collections remain an untapped resource struggling to justify long-term funding and establish productivity [41]. However, aligning these collections with specimens and data from *disease-oriented biobanks for epidemiology* creates an ideal platform to compare prospective pre-diagnostic specimens and exposure assessment with specimens collected during the peri-diagnostic period. This approach is particularly suited to identifying biomarker-based molecular tests from non-invasive specimens to facilitate the cost-effective screening and diagnostic tests [42] to find tumours at an early stage when treatment is most likely to be effective [43].

A sub-group of the larger *population biobank* will eventually become case subjects within the *disease-oriented biobanks for epidemiology*. The compatible data and biospecimen collection facilitated by integrating these distinct models of biobanking elevates the validity of the downstream research. The detailed lifestyle –related factors including diet, occupation and medication use are often collected prospectively thereby eliminating recall bias that can undermine traditional case–control studies. Furthermore, strategic distribution of specimens to support external research, such as carefully matched control subjects, establishing biomarker baseline variation levels and deriving non-clinical endpoints are all productive applications of population biobank specimens prior to the accumulation of clinical endpoints.

Although *disease–oriented general biobanks* and *population biobanks* are generally considered to be at the polar extremes of the biobanking spectrum, both approaches can benefit from the respective strengths of the other. Since *disease–oriented general biobanks* are generally embedded within clinical systems, the collection of peri-diagnostic specimens is more easily accomplished. Consequently, biobanking initiatives at the intersection of these approaches provides data quality and validation opportunities that are not available if these entities are isolated.

The ACRB has invested in the integration of all three biobanking approaches. The *population biobank* is represented by *Alberta's Tomorrow Project*. The standard operating procedures for blood collection, processing and storage have been harmonized with the population-based prospective cohorts of selected cancers which represent the *disease-oriented biobanks for epidemiology*. The *Breast Cancer to Bone (B2B) Metastasis Research Program*, the *Alberta Moving Beyond Breast Cancer (AMBER) Cohort Study* [44], the *Colorectal Cancer Lifestyle and Environment in Alberta Research (CLEAR) Study* and the *Prostate Cancer Active Surveillance Clinic (RAC5)* projects all operate their collections and storage through the common infrastructure provided by the ACRB. By integrating the common biobanking operations, the ACRB has evolved from an opportunistic "convenience collection" of mainly tumour tissue biospecimens and associated data to a comprehensive cancer-focused biobank incorporating population-based patient cohorts for observational studies consisting of the tumour groups which represent over half of all newly diagnosed cancers in Alberta. By adopting this new strategy, the ACRB simultaneously addresses the immediate and future needs of specific research programs, leverages biobanking resources to facilitate economical, productive and hypothesis-focused banking and builds a unique inventory of cancer-related biospecimens comprising well-characterized prospective cohorts.

8.5 Population Metrics in Biospecimen Distribution

Since the role of biobanks is to supply biospecimens for research, the most critical point at which to apply population metrics to biospecimen collections is prior to biospecimen distribution. Analyzing biospecimens from an incompletely described population limits the opportunity to

generalize the research findings or specify the sub-groups to which they might genuinely apply. Biobanks must provide full transparency regarding participant selections and the collection of the biospecimens since this is an essential component of the fundamental comparison of every observational study [15]. To help achieve this, biobanks should offer investigators a description of the cohort of biospecimens distributed in the context of the source population, in a similar way that clinical trials participants are represented according to the CONSORT guidelines [45, 46]. A researcher requesting biospecimens typically includes a set of parameters pertaining to the disease or subjects that are necessary to address a specific research question. For example, a researcher may request serum from HER2-negative breast cancer patients with stage I or II disease. Patients who fit all these criteria are said to be "eligible" for a researcher's study. To determine if a set of biospecimens is representative of the source population, biobanks need to formally compare important patient factors (e.g., age, cancer stage, tumour grade, etc.) between the eligible biospecimens and the source population from which they were collected. Cancer registries are immensely useful for the provision of these data but, in absence of comprehensive registries, the minimum standard should be to report the aggregate data for a given collection site with possible comparisons with equivalent population-based sources. Significant deviations between these factors for the biospecimen donors and the source population may be justifiable but must be fully disclosed. Providing these data will improve the efficiency of downstream research by reducing the number of studies that are based on fundamentally flawed collections. However, it is important that the costs associated with obtaining and distributing these data are incorporated into core biobanking activities and adequately resourced. Biobanks must take responsibility to inform biospecimen recipients and emphasize the observational nature of the materials they are distributing so that any limitations on subsequent interpretations can be adequately considered.

8.6 Biobanking Networks

The overall number of human tumour tissue biospecimens used per research publication has increased significantly over the past 20 years [47]. However, it is not always possible for a single biobank to satisfy a request for biospecimens from a cancer patient cohort that is sufficiently large while still representative of the source patient population because of the complex and heterogeneous nature of the disease and differing collection priorities. Moreover, individual biobanks may also struggle to satisfy investigator requests when study designs require very rare forms of cancer, have narrow and specific cohort requirements and/or sample sizes ranging from 2,000 to 50,000 subjects [47]. In response, groups across North America and Europe have formed networks of biobanks to overcome sample size limitations and provide the high-quality biospecimens and associated data that are required for robust biomarker discovery and validation. These groups include the Organization of European Cancer Institutes (OECI) TuBaFrost consortium in Europe, the Office of Biorepositories and Biospecimen Research (OBBR) in the USA and the Canadian Tumour Repository Network (CTRNet) in Canada.

The success of any biobanking network depends on its capacity to supply sufficient numbers of high quality biospecimens in a timely manner. Each biobanking network strives to establish the confidence of the research community based on the quality of their biospecimen collections. Consequently, biobanking networks have been at the forefront of efforts to define biospecimen quality metrics and establish procedures to minimize pre-analytical variation from biospecimen handling [48]. These measures include the adherence to best practices and standard operating procedures for biospecimen collection, processing and storage such as those developed by CTRNet [49]. In addition, biobanking networks may offer a more stringent system of auditing such as the certification program offered through CTRNet [50].

Advocating for increased transparency of participant selection and the distribution of biospecimen population metrics represents a significant opportunity for existing biobanking networks to improve the relevance of their collections and expand the scope of biospecimen quality to include these components. By adding the reporting of population metrics to biospecimen distributions, biobank networks can help reduce a potential source of pre-analytical bias that may be introduced by participant selection. An additional benefit of large biobanking networks is that even if individual collections are not representative of disease burden, derived cohorts can be formatted from collections spanning the entire network using the frequency matching approaches used in case–control and cohort studies, to better represent disease burden for downstream research [51].

8.7 Key Consideration for Biospecimen Quality

To date, the vast majority of biospecimen quality-related research has focused on the variables associated with the lifecycle of a biospecimen that determine specimen quality [48]. The collection, processing, storage, handling, distribution and final analyses have all been the subject of intense scrutiny and the associated metric for each aspect are typically proposed as a measurement of quality. Adopting optimal and standardized practices for each of these steps is essential for preserving the integrity of the biospecimen and the validity of downstream research. However, even the most impeccably collected, processed, stored, handled, distributed and analyzed specimens can still provide misleading results if they do not represent the disease or population from which they were selected. Participant selection is a potential source of pre-analytical variation (Fig. 8.2). Consequently, considerations of biospecimen quality must include an assessment of participant selection.

8.7.1 Pre-intervention Biospecimens

The timing of the collection of biospecimens, along the natural history of a disease or the diagnostic and treatment trajectory of a patient, is an important consideration for the suitability of those specimens for the downstream research applications. This timing is of particular importance in the context of cancer patients for whom treatments, including surgery, radiation therapy and systemic therapies, can alter local and systemic factors. The analysis of blood collected from individuals with a confirmed cancer diagnosis allows for the assessment of secreted molecules released into the blood stream by cancer cells or other cell types associated with the tumour microenvironment. Indeed, the derivation of diagnostic and prognostic signatures from human blood samples offers enormous potential to achieve the goals of personalized medicine for cancer control [42]. Blood sample collection is a relatively non-invasive, cost-effective and easily accessible procedure and analysis of the serum and plasma blood fractions can reveal informative signatures from nearly all tissues and organs [42].

There is surprisingly little methodological literature concerning the impact of surgical intervention on serum and plasma protein biomarkers. However, the literature that does exist is compelling [52–56]. As might be expected, the surgical removal of a primary tumour results in a significant decrease in the concentration of biomarkers that are associated with tumour progression such as the extracellular domains of HER2 and EGFR, CA 15–3, CEA and CCSA-2 (Table 8.2) [52, 54]. In contrast, the concentration of acute-phase proteins and associated circulating cytokines, such as VEGF, IL-6 and CSF1, significantly increase as a result of surgery in breast and colorectal cancer patients, despite already elevated pre-operative levels relative to healthy controls [60]. Furthermore, this elevation is also dependent on the extent of surgical intervention,

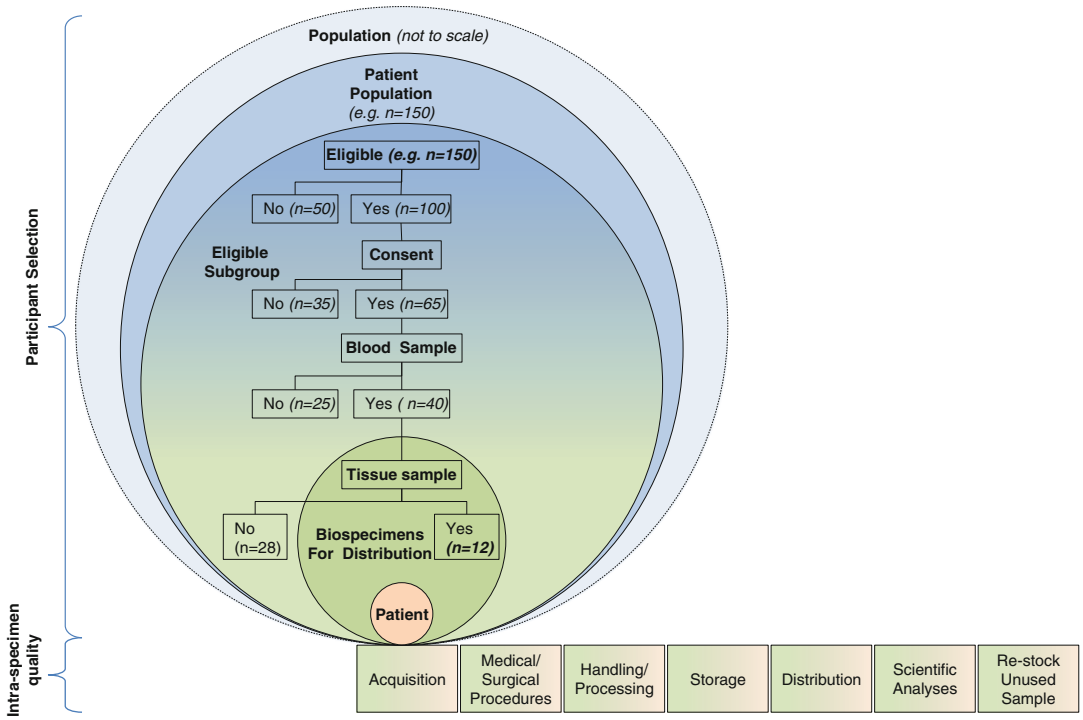

Fig. 8.2 Sources of pre-analytical variation in biospecimens. To date, assessment of specimen quality has focused almost entirely on intra-specimen factors. The extent of participant selection and representativeness must also be considered when assessing biospecimens collections. In this example, requiring matched blood and tissue specimens, 100 are eligible from a pool of 150 patients, consent is obtained for 65, blood is available for 40 and tissue for 12. Consequently, in downstream analyses of matched samples, the disease would be represented by 12 % of eligible patients. It is essential that the baseline characteristics of those 12 patients are compared to the eligible patients to address the potential impact of selection bias on downstream research

associated tissue damage and the timing of post-operative sample collection [55, 59, 61, 62]. Therefore, removal of a primary tumour induces additional inflammation that is evident as a systemic response that changes the profiles of protein blood biomarkers [63].

Significant changes in blood protein biomarkers levels have been reported between pre- and post-intervention with systemic and local non-surgical therapy (chemo- and radiotherapy) in breast and colorectal cancer patients. Unsurprisingly, these therapies typically decrease the concentration of circulating tumour progression proteins such as extracellular HER2 and CEA in breast and colorectal cancer patients, respectively [64–66]. Systemic therapy also has a significant impact on the concentration of circulating angiogenic factors and cytokines such as VEGF and IL-6 [67–69]. Tumour tissue specimens should also be obtained prior to systemic therapy, ideally at diagnosis or at the time of the definitive resection, because non-surgical treatment can also alter intra-tumoral and systemic biomarkers levels [21]. The effects of non-surgical interventions on cancer patients and biospecimens are complex, are dependent on the types and combinations of pharmaceutical and/or radiation therapies administered and can significantly affect biomarker concentrations.

In addition to changes in circulating protein biomarkers, both surgical and non-surgical treatment interventions can disturb the nucleic acid and metabolic profiles of cancer patients. The impact of treatment on circulating cell-free DNA in cancer patients has been known for many years [70] and more recent studies have demonstrated alterations to circulating miRNA biomarkers [70–72]. Similarly, when the metabolomic pro-

Table 8.2 Significant changes to specific blood protein analytes in response to surgical intervention in breast and colorectal (CRC) cancer patients

Disease site	Mean sampling to intervention interval (days)		Sample size (n)	Protein analyte	Significant increase or decrease ($p \leq 0.05$)	Reference
	Pre-intervention	Post-intervention				
Breast	NR	NR	89	HER2 BCL2 CA 15–3 CEA	– – – –	[52]
Breast	7	45	26	TGF-β	–	[53]
Breast	1	1	119	HER2 EGFR	– –	[54]
Breast	NR	NR	52	VEGF	+	[55]
Breast	0.3	1	79	IL-16 IL-6 CSF1 THBS2 HER2 VEGF IL-7 FasL	+ + + + + + + +	[56]
CRC	3	7	106	CCSA-2	–	[57]
CRC	NR	7	50	TGF-β	–	[58]
CRC	0	1, 3	139	VEGF	+	[59]

files of breast cancer patient sera are examined, changes in analyte abundance are observed between blood collected before, during and after surgical and/or systemic treatment interventions [73, 74]. Consequently, the timing of biospecimen collection, with respect to interventions, should be carefully annotated and fully disclosed to biospecimen recipients.

If resources allow, both pre- and post- intervention blood samples should ideally be collected for all participants. This strategy would facilitate the assessment of biomarkers associated with the diseased state and the impact of treatment. However, due to the complexity of healthcare delivery and the resource-intensive nature of population-based biobanking operations, there is a necessary trade-off between focusing resources on baseline (pre-intervention) biospecimens and the additional time-points. Typically, the over-riding priority is to obtain representative samples from the largest possible cohort of participants, especially for biobanks that are focused almost predominantly on DNA as the sole biospecimen type. The paucity of literature investigating pre- and post-intervention biomarker levels indicates that these analyses have not been a primary priority for many biobanks. Convenience and genetically-focused biobanks have sustained the relatively unchallenged collection of post-intervention blood samples.

The interval between diagnosis and treatment, for many diseases, is often variable and can be very short. For many diseases, including many cancers, there are no clear national or international guidelines that define acceptable intervals between diagnosis and treatment. This period varies by several factors including jurisdictions, healthcare organizations, type and stage of disease diagnosed [75, 76]. For example, prostate cancer patients diagnosed with a favorable-risk disease often undergo extended periods of active surveillance prior to treatment, while colorectal cancer patients with an emergent presentation may be scheduled for surgery on the day of diagnosis [77, 78]. This unpredictable recruitment window presents substantial logistical challenges for any biobank attempting to collect pre-intervention biospecimens. However, in light of

the impact of interventions on biomarker levels described above, overcoming these challenges is essential if the most relevant and valuable specimens are to be obtained. Furthermore, since disease severity will often influence the urgency of treatment, biobanks seeking to collect population-based pre-intervention biospecimens must implement mechanisms to accomplish patient recruitment and biospecimen collection within the minimum interval if the systematic exclusion of more severe cases is to be avoided and population-based recruitment maintained.

8.8 Population-Based Tissue Collection

Two very different storage approaches currently account for most tissue samples that are collected and stored, long-term, for research purposes. The vast majority of human biospecimens have been collected and stored as formalin-fixed, paraffin embedded (FFPE) tissue for diagnosis and therapy; only a fraction of these collections were used for quality assessment, educational and research purposes [2, 79]. Archiving specimens as FFPE tissue blocks has been the standard practice in healthcare since the 1950s. The collection and storage of fresh-frozen samples has been largely restricted to research-focused institutions with only very recent and limited clinical applications. Both methods have strengths and weaknesses and suitability for different downstream research uses.

The formalin fixation process was developed to preserve the histopathologic features of tissue for diagnostic purposes; the longevity of the archived clinical samples is a testament to the effectiveness of the process in this regard. However, the fixation process also modifies cellular macromolecules and, until very recently, rendered these specimens unsuitable for many of the sensitive molecular and genomic characterization technologies [80, 81]. Fortunately, the almost ubiquitous availability of FFPE tissues in healthcare settings prompted researchers to develop sophisticated techniques to expand the research applications of this rich resource. Techniques that had previously only been possible with fresh-frozen biospecimens are now reliably conducted on FFPE tissues. Indeed, nucleic acids and proteins are now routinely extracted from archived FFPE blocks and successfully utilized for whole genome gene expression and proteomic analysis, respectively [82–85]. The development of tissue micro-array technology since 1998 [5, 86] has contributed to the expansion in analytical techniques available for FFPE tissue substrates and is reflected in the significant increase in the relative proportion of research studies using FFPE tissues alone and the concomitant decrease in the use of fresh-frozen tissues [47].

While the use of fresh-frozen tissue in research studies is declining, it remains an extremely valuable resource for research because the biochemical functions that are destroyed by formalin fixation are typically well preserved in fresh-frozen tissue specimens. Consequently, fresh-frozen tissue can still support a more comprehensive interrogation of tissue biology. However, fresh-frozen specimens are generally only collected after the tissues necessary for essential diagnostic work-up and clinical care have been collected. This secondary prioritization means that, for small tumours, it is often not possible to collect fresh frozen specimens and larger tumours are necessarily over-represented in fresh-frozen tissue collections. The selection bias introduced by tissue abundance may be particularly serious for anatomic sites in which small (<1–2 cm) tumours are relatively common, such as the breast.

Tissue collections are more susceptible to selection bias than blood collections because of the necessary invasiveness of the procedures, the limitations of tissue abundance and any circumstance that may diminish or obviate the collection of tissue, such as neoadjuvant chemotherapy, unresectable tumours or unknown primary tumours. FFPE is a better substrate for the implementation of clinically relevant predictive markers and personalized medicine because the collection and processing infrastructure is already established in healthcare settings and the collection and processing of these tissues is prioritized

most highly. However, the collection and storage of fresh-frozen tissues remains a worthy investment because they are ideal for assessing biochemical integrity and deriving reference standards for corresponding biomarkers measured in FFPE specimens.

8.9 Sustainability of Population-Based Biospecimen Collection

Despite acknowledging the limitations of a substantial proportion of the existing biomarker literature and the contribution of unsuitable and highly selected biospecimens in those shortcomings, there has been little advocacy, within the biobanking community, to change the way research participants are identified and biospecimens collected. This inertia may be attributable to the higher costs and logistical challenges associated with comprehensive population-based ascertainment and recruitment. The relative ease of opportunistic collections and the disregard for the observational nature of biospecimens has created little incentive to make participant selection or robust population metrics a significant priority for biobanks. However, since sustainability is a major concern for 41 % of biobanks [5], adopting these approaches, integrating the three biobanking models [40] into a comprehensive system and offering biospecimens that address the potential weaknesses of many existing collections, represents an opportunity to distinguish those biobanks and increase revenue potential and sustainability (Fig. 8.1). Aligning the epidemiologic approaches of *population biobanks* and *disease-related banks for epidemiology* with the clinically-focused and embedded biobanking solutions of *general biobanks* for the collection, processing and storage of human biospecimens creates efficiencies and synergy through cost sharing, human resources and shared expertise. These strategic partnerships benefit the whole biobanking and research enterprise by harmonizing specimen and data collection, storage, tracking and distribution. The expertise provided by observational researchers enhances the study design and patient recruitment aspects of the biospecimen collection strategy.

Observational research studies typically require a specific subset of a patient population that fit a set of eligibility criteria designed to answer specific research questions. Grant-funded prospective observational studies can be nested within comprehensive cohorts recruited by the overall biobanking enterprise with consent to be contacted for future research obtained at the time of consenting for biospecimen collection. This explicit consent component enables the biobank to facilitate the recruitment of participants for downstream studies that require participant contact for additional data collection. The biobank can at least partially recover costs of recruitment for those participants involved in the downstream studies whilst retaining a proportion of the biospecimen inventory for additional research uses.

Our experience, in Alberta, indicate that the majority of the cost associated with obtaining and banking a human blood donation is incurred by the process and labour costs of identifying a cancer patient and obtaining their informed consent for a biospecimen collection. Eligibility for downstream research often cannot be determined until consent has been obtained. Therefore, by investing the additional resources to collect biospecimens from all patients who provide informed consent, not only those patients eligible for specific priority research studies, a biobank can potentially establish a comprehensive population-based biospecimen collection (Fig. 8.3). This approach increases the likelihood that the biobank can accommodate future biospecimen requests because its inventory is not restricted by the inclusion criteria of the associated research studies. Furthermore, by processing the blood collections into a large number of aliquots, a biobank may optimize the research use of biospecimens by releasing specimens to specific epidemiological researchers while retaining a portion of the collected inventory for distribution to external researchers.

This approach to biobanking is novel but also consistent with the study designs advocated by the PRoBE guidelines [24] and the leveraging of cohorts proposed by Ransohoff [87]. New

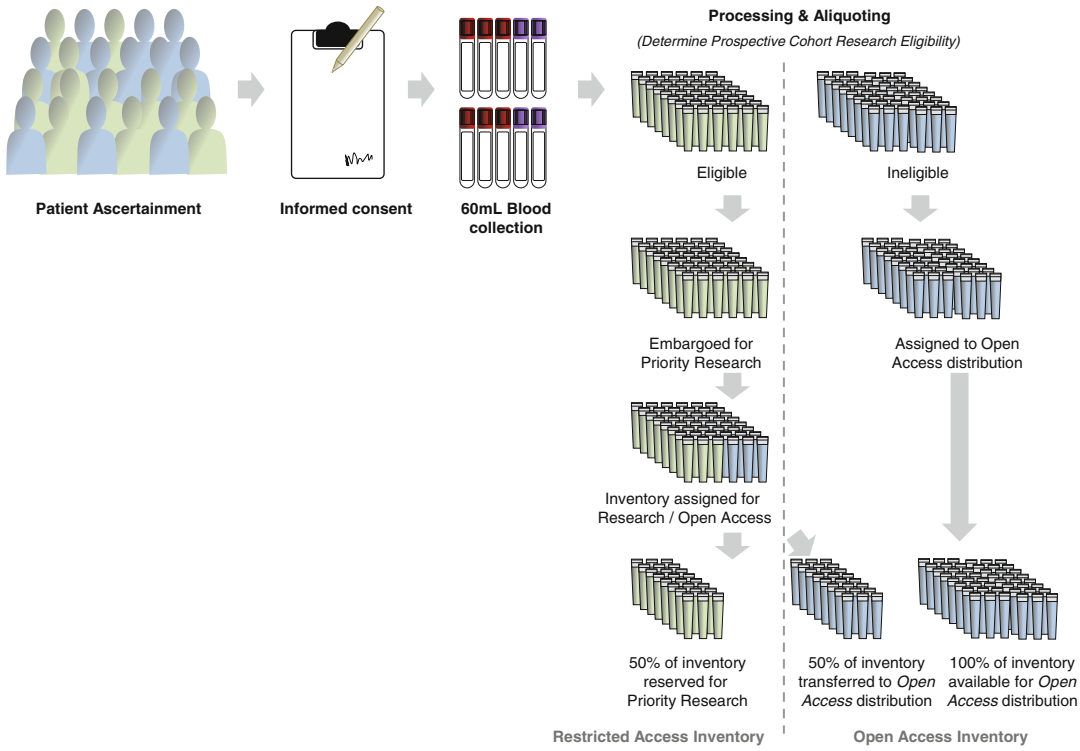

Fig. 8.3 Optimizing recruitment and biospecimen inventory allocation to leverage prospective cohorts within a biobanking infrastructure. Building population-based prospective patient cohorts is extremely resource intensive. The majority of the cost is incurred during patient identification and obtaining informed consent. If informed consent and collection of biospecimens is sought from all patients (for priority anatomic tumour sites), and sufficient inventory collected, participants can be selected for downstream investigator-initiated studies (according to study-specific eligibility) and recruitment costs partially recovered. This system facilitates prospective study designs, ensures that specimens are used in predefined research but also available for additional, originally unforeseen, studies

research questions can be readily answered by "piggybacking" on existing well-designed cohort studies where the resource-intensive biospecimen collection is already complete [13]. Indeed, there are several examples where investigators have successfully leveraged biospecimen collections from existing studies, primarily RCTs, to address new research questions [87]. One such example involves the use of a biobank created as part of the National Cancer Institute's Prostate, Lung, Colon, Ovary (PLCO) RCT [48]. The original trial set out to determine if a blood sample, collected shortly before diagnosis, is capable of detecting asymptomatic cancer by collecting serial blood samples from a healthy population followed for the development of cancer. Leveraging the serial blood sample collections from a subset of ovarian patients collected for the existing well-designed PLCO trial, researchers tested a new hypothesis that a blood-based proteomics test may be used for screening early ovarian cancer with greater sensitivity and specificity than the leading CA125 biomarker. Researchers used only the observational elements of the PLCO study, rather than the randomization of the RCT, to rapidly and efficiently disprove their original hypothesis; the availability of biospecimens from the RCT eliminated the lengthy and expensive but necessary biospecimen collection component of their research design [87]. The lesson learned from the above example, testing of new hypotheses by leveraging existing cohorts, can be extended to cancer biobanks and pre-intervention blood specimens collected from

population-based patient cohorts. The critical component is that participant selection was appropriate and well-documented.

Numerous biobanks report an underuse of their biospecimens; over-supply of specimens outpacing demand and poor marketing are both cited as major contributing factors [88]. Therefore, it seems that the conclusions of the original NCI internal and external review of biospecimen resources remains at least partly valid today [2, 89]; there is an abundance of biospecimens available, but they do not adequately meet the needs of researchers [88]. While approximately 180 commercial biobanks operate in the United States alone, no single company holds more than a 3 % share of the global biobanking market. The biobanking industry is highly fragmented and there is considerable variability of biospecimen quality within and between individual biobank collections. However, the projected continued growth in the value of the global biobanking market clearly demonstrates that the demand does exist for the right products [5]. Therefore, individual biobanks or networks of biobanks can take the opportunity to gain a global competitive advantage by offering evidence of exceptional biospecimen quality and/or novel products that surpass existing metrics of human biospecimen commodities.

8.10 Conclusions

Biomarker research and personalized medicine have traded on their undoubted potential to transform healthcare and must now start delivering tangible advances. Biobanks have an essential role to play in improving the standards of biospecimens and data quality. Since biobanks are a common source of biospecimens for translational research, biobanks must be the proponents for change; translational researchers will only embrace these observational methods and reporting standards if their impact can be demonstrated and their value clearly understood. We propose that biobanks provide population metrics for all biospecimens that they distribute and ensure that researchers report these data in resulting publications. Biobanks must also advocate for such data to be a minimum expectation for journal editors and peer-review. The biobanking community must establish a global dialogue to ensure that the principles of observational research are appropriately applied to biobanking activities and downstream research. We also propose that considerations of biospecimen quality go well beyond assessments of degradation and integrity to encompass the selection of participants and timing of collections. Clearly, despite significant progress in the field of biobanking, many issues remain to be addressed and opportunities exploited. Embracing the observational nature of biospecimens and changing biobanking practices to improve study design represent a pivotal opportunity to improve biobanking in the twenty-first century.

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Challenges in Developing a Cancer Oriented-Biobank: Experience from a 17 Year-Old Cancer Biobank in Sao Paulo, Brazil

9

Antonio Hugo Jose Froes Marques Campos and Fernando Augusto Soares

Abstract

Brazil and Latin America will face a cancer epidemic in the coming years. Efforts towards cancer prevention, early detection and treatment must be associated with active research that helps understanding the geographical variations of this disease. The creation of cancer-oriented biobanks should be part of this strategy. This article outlines the challenges of establishing a cancer-oriented biobank at the A. C. Camargo Center, a private, non-profit institution located in Sao Paulo, Brazil. We analyze important issues related to the day-to-day operations of the biobank within an institutional and national context, as well as the lessons learned over the years. It is hoped that the information contained in this paper will be useful for the development of other biobanks in Brazil and other countries in Latin America.

Keywords

Biobank • Cancer • Challenges • Regulations • Operational issues • Brazil • Latin America

9.1 Introduction

Latin America has experienced a marked socio-economic transition in the last decades [1]. Despite the socioeconomic inequality that persists in the region, a rapid process of population aging is taking place in most countries. Data from 2010 shows that 5 % of the Brazilian population are 60 years old or older (which translates into 9.5 million people). By 2050, it is expected that Brazil will have about 58 million people with 60 years old or older. This ageing phenomenon,

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coupled with a more widespread adoption of western diet and lifestyle, has already impacted the ranking of death causes. In Brazil, cancer is now a major cause of death, second only to cardiovascular diseases [2]. Data from the Brazilian Ministry of Health show that the annual cost of cancer care parallels the ranking of death causes. Hospitalizations for cancer care have reached a cost of approximately US$ 250,000,000/year and are expected to rise in the coming years [3].

The same scenario has been observed in other Latin American countries [4–6], which are required not only to increase the efforts towards cancer prevention, early detection and treatment, but also to promote cancer research that helps understanding the geographical variations of the epidemiology and molecular aspects of this disease.

In this regard, the last 20 years have witnessed a rising interest in the potential value of creating the so called tumor banks, facilities dedicated to collect, store and process human biological samples (e.g., neoplastic and non-neoplastic tissue, blood, urine, saliva), and together with associated clinical and pathological data, distribute them to investigators dedicated to cancer research [7]. Well-established biobanks have the potential to provide human biological samples (and associated data) with the quality required to perform research using next generation sequencing techniques [8]. Despite the growing number of biobanks being created in Brazil and other Latin American countries, currently few of them have reached a stage in which they can actively contribute to cancer research.

In this paper, we describe the experience and challenges faced in developing the A. C. Camargo Biobank, a cancer-oriented biobank located in a tertiary cancer center in Sao Paulo, Brazil, taking into consideration the contextual backgrounds of our institution and the Brazilian society. By analyzing the trajectory of this 17-year old biobank, we aim to provide useful insights that can be used as a guideline for the development of other biobanks in developing countries.

9.2 Background

The A. C. Camargo Cancer Center is a civil, private, non-profit, closed staff hospital established in 1953 and maintained by Fundacao Antonio Prudente [9]. The origins of its Biobank can be traced back to 1997 (Fig. 9.1), when the hospital started to collect fresh frozen tissue (tumor and non-neoplastic counterpart) and blood to be used in what came to be known as the project "Genoma Humano do Cancer" (Human Cancer Genome Project), launched in 1999 with grants from the State of Sao Paulo Research Foundation/FAPESP [9, 10]. As such, this tumor bank could be classified as an oligo-user biobank (a collection connected to a research group, usually within the same institution and planned for a series of primary and secondary research questions) according to Watson & Barnes' human research classification schema [11]. However, the Tumor Bank evolved to support multiple research projects proposed not only by investigators from within the hospital, but also from other institutions (national and international) through the establishment of collaborations (Fig. 9.2). In 2004, another level of complexity was added, with the creation of a Bank of Macromolecules to centralize macromolecule extraction and purification in order to distribute aliquots to research projects. This new structure (detailed in Fig. 9.3 and called "A C Camargo Biobank") would be better classified as a poly-user biobank according to the same classification schema [11].

It is important to note that, during this time, no specific regulatory framework on biobanking was in place. Only in 2005 the National Health Council (CNS) of Brazil approved Resolution CNS 347/05 regulating the use of human biological materials in research projects [12]. This Resolution was implemented based on the premise that scientists would collect human biological material during the development of a specific research project. As such, it did not kept up with the evolution that had occurred in the A. C. Camargo Biobank, which by 2005 was already

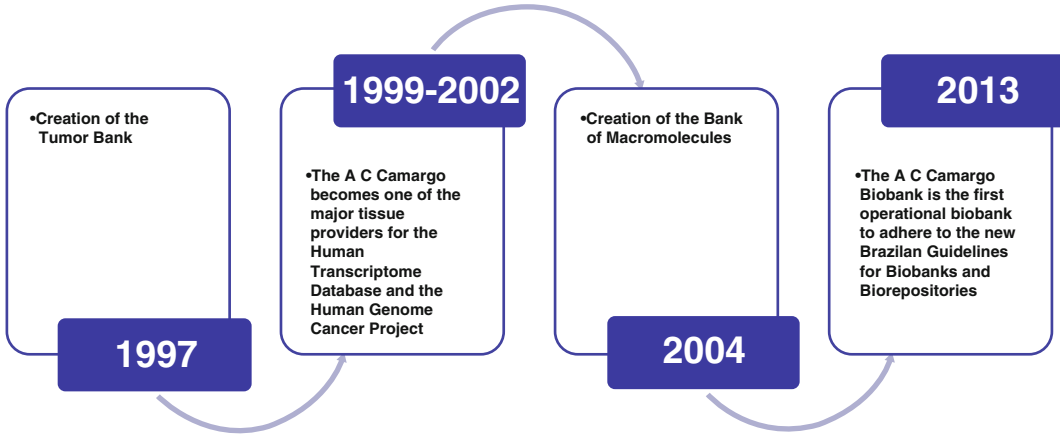

Fig. 9.1 Origin and evolution of the A. C. Camargo Biobank

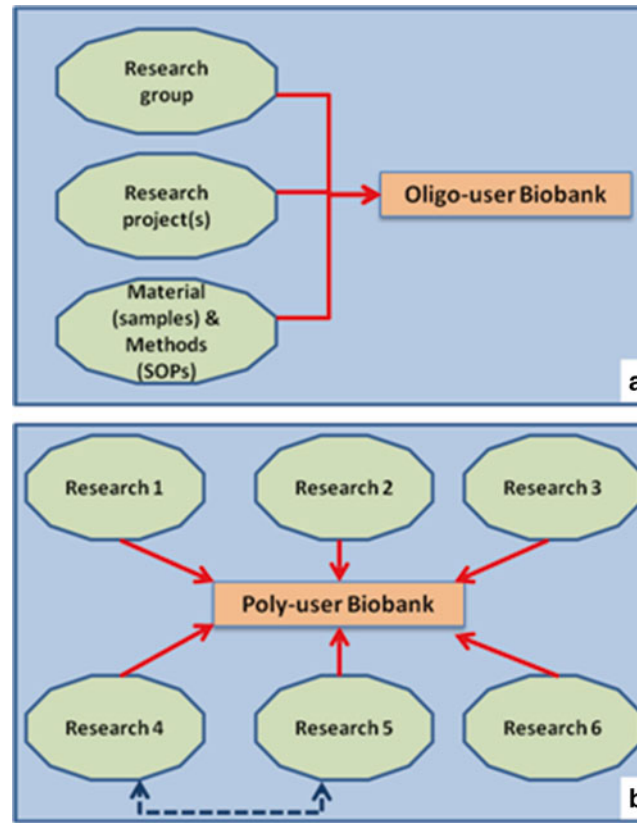

Fig. 9.2 Evolution of the Tumor Bank from a oligo-user biobank dedicated to provide samples for a series of research questions made by a research group (**a**), to a poly-user biobank dedicated to support multiple and frequently independent research projects (**b**)

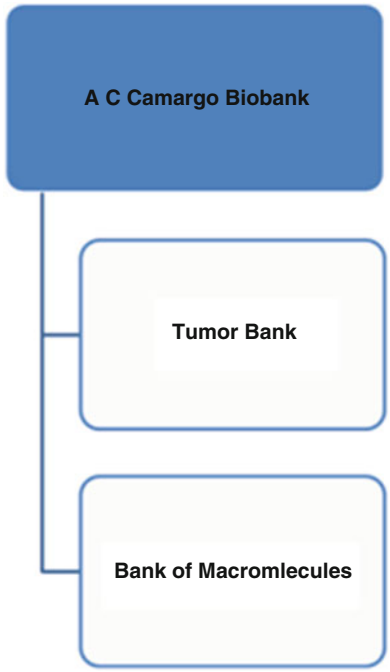

Fig. 9.3 Current structure of the A C Camargo Biobank. The Tumor Bank is responsible for collecting processing and storing tissue samples, blood and other fluids. The Bank of Macromolecules is responsible for macromolecule extraction, quantification and quality analysis before distributing aliquots to investigators

functioning as a poly-user biobank intended for future undefined use.

This model of biobanking would only be acknowledged 4 years later, when the Ministry of Health (MS) formed a working group, comprising members of the Brazilian Research Ethics Commission (CONEP, a branch of CNS), bioethicists, researchers, and biobank managers. The technical, legal, and ethical issues of biobanking were addressed by the group during a 2-year period (2009–2010), and resulted in a new regulation (MS Ordinance No. 2201) that was implemented in 2011. At the same time, the CNS published the Resolution CNS 441/11, replacing Resolution CNS 347/05. The rationale for these new regulations is reviewed by Marodin et al. [13]. Briefly, both regulations now acknowledge the existence of systematic collections of human biological material for future use in research ("biobanks") and time-limited collections of biological material by an investigator (or group) for a research project whose purpose is already known ("biorepository"). It should be noted that this new regulatory landmark with the definitions for a biobank or a biorepository came into force at the same time of the publication of the classification schema proposed by Watson and Barnes [11]. Therefore, the definition of a biobank in the Brazilian regulatory landmark would apply to a poly-user biobank, while a biorepository would apply to a mono-user biobank or an oligo-user biobank.

The demand shift (the need to attend multiple and frequently unrelated future research projects), together with the recent regulatory changes related to the use of human biological samples for health research, raised operational and ethical issues discussed herein.

9.3 Operational Issues

9.3.1 Standard Operating Procedures (SOPs)

During the first phase of the A. C. Camargo Biobank (in which only the tumor bank was operating), tissues and blood were provided directly to investigators without further processing. This banking model was considered adequate for the first years of operation because it allowed adopting uniform procedures for tissue collection, processing and storage [14]. Among other things, this decision made easier for professionals from departments closely involved with the biobanking activities (e.g., pathologists, residents) to perform tissue collection during their routine, and facilitated the implementation of quality control checks for critical phases of the process (see below in Sect. 9.3.2).

The decision to create a central facility for macromolecule extraction (Bank of Macromolecules) was based on the knowledge that the samples collected were finite and, once directly provided for a specific research project, their potential value for other applications would disappear [15]. However, the biobank first identified the most in-demand molecules for investigators in order to maximize the potential value of

samples without adding to much complexity to the system.

Therefore, initial protocols favored the simultaneous isolation of RNA and DNA, for example using TRIzol® reagent (Invitrogen Corporation, CA, USA). Over the years, and according to new demands made by investigators, new kits were introduced (e.g., for miRNA isolation or for use in automated macromolecule extraction). It is expected that the revolution of next generation sequencing will impose new demands on the activities of the Bank of Macromolecules. Again, to meet these challenges, the biobank will have to balance the potential benefits and costs of introducing new levels of complexity to its operations in order to maintain the policy of centralizing the extraction and distribution of macromolecules.

Another operational issue was the adoption of optimal standards of cryopreservation. Although many biobanks typically use −80 °C cryogenic freezers to store tissues, since the beginning the tumor bank decided to adopt equipments operating at temperatures below the glass transition of water (around −130 °C). In 2007, a document released by IARC-WHO confirmed that −130 to −150 °C was de desired temperature range for the storage of tissues [7]. A recent review by Hubel and collaborators concluded that "storage temperatures below −135 °C appear to be necessary in order to preserve a wider variety of biomarkers (including viability)" [16]. Our group has also shown that 80 % of all RNA molecules extracted from tissues stored for up to 7 years at −140 °C were suitable for use in research projects [15]. From our experience, and the data available in the literature, we believe that biobanks using prolonged periods of storage for their tissue samples should consider adopting storage temperatures below −130 °C, regardless of using liquid nitrogen cryotanks or mechanic cryofreezers.

9.3.2 Quality Control Issues

The validity of research results is heavily dependent on the quality of samples. Being primarily a tumor tissue bank, a cultural change was needed in the Department of Pathology in order to integrate the biobanking activities within the diagnostic routine [17, 18].

In the first phase of the evolution of the A. C. Camargo Biobank, this meant ensuring that the primary tumor samples collected from surgical specimens had adequate representativeness. Whenever possible and appropriate, correspondent non-neoplastic tissue and metastatic tumor tissue also had to be collected. Later, it required that the samples collected for cryopreservation were processed in a timely manner. With the collaboration from other professionals (surgeons, nurses, technical assistants), cold ischemia times had to be recorded. In order to assess the consistency in these activities, tissue representativeness and cold ischemia times began to be continuously monitored and reported to the professionals of the Department of Anatomic Pathology.

The involvement of different professionals may be easier in a closed staff private hospital with a unified institutional policy. However, in institutions with different internal policies (e.g., universities in which the different departments are relatively independent, or in open-staff hospitals) the collaboration of such professionals may be difficult to obtain. Regardless of the strategies adopted to overcome internal policy issues, one of the cornerstones for success in biobanking is institutional commitment, not the isolated effort of one individual or a single group.

Another issue was the integrity of RNA aliquots extracted from tissue samples and stored over time at −80 °C. As investigators continuously request RNA aliquots stored at the Bank of Macromolecules to conduct their research, the RNA extracted from tissue samples is diluted in an aqueous solution before cryopreservation, which can induce degradation by endogenous RNases. Our group has shown that diluted RNA aliquots need to be stored at higher concentrations in order to avoid degradation. The experience gained over the years and the data available in the literature show that biobanks should implement and follow quality control protocols to ensure that the quality of macromolecules provided to investigators is adequate for research [15].

9.3.3 Long-Term Sustainability

Adequate staffing, primary and back-up equipments, maintenance contracts and consumables make the cost of setting up and maintaining a tumor bank the greatest barrier faced by any Brazilian or Latin-American institution, especially those that need to start from the beginning. For example, the annual operating budget of the A. C. Camargo Biobank has been estimated in approximately $ 300,000 USD. Because cost recovery fees are not sufficient to balance the budget, constant effort is made to attract funding from different sources. State and Federal grants help keeping cost-recovery fees at reasonable prices while allowing the biobank to expand and modernize its structure. Nevertheless, long-term institutional support has been essential in maintaining the biobank and indirect measures of success need to be regularly updated. Although the number of donors and specimens is important to assess the success in getting patient support, the number of projects that were granted access to the biobank resources, the number of specimens accessed by investigators and the number of publications are more important to assess the usefulness of the biobank. Data from the last 4 years show that the number of research projects requiring access to samples has been constantly growing (Fig. 9.4), but the number of new samples collected exceeds the number of stored samples required by investigators. Even considering that the samples are not required in the first years because relevant follow up data is not available, new strategies to foster the rational use of the biobank need to be implemented. Likewise, a previous work from our group showed that a constant effort must be made to ensure that investigators acknowledge our biobank in their publications [10].

9.4 Ethical Issues

In 2011, there was an increase of 13 % in the number of surgeries carried out at the A. C. Camargo Cancer Center compared to 2010 (from 7,378 to 8,342 procedures) [9]. Hypothetically, all patients submitted to these procedures were eligible for tissue banking, provided that there was valuable and available enough tissue to be collected for banking and research without compromising pathological diagnosis. As observed by Hewitt [19], appropriate counseling is a time consuming process, patients may not be readily available or may not be prepared to make a conscious decision about donating their material for research in the pre-operative period. In fact, data from 2011 until February 2014 (Fig. 9.5) show that the rate of patients who refused to donate sample for banking and research at the A C Camargo Cancer Center did not exceed 5 %. A more alarming finding is the rate of patients who did not decide on whether to donate or not their samples prior to surgery, which started to rise in July 2011. These patients would have to be

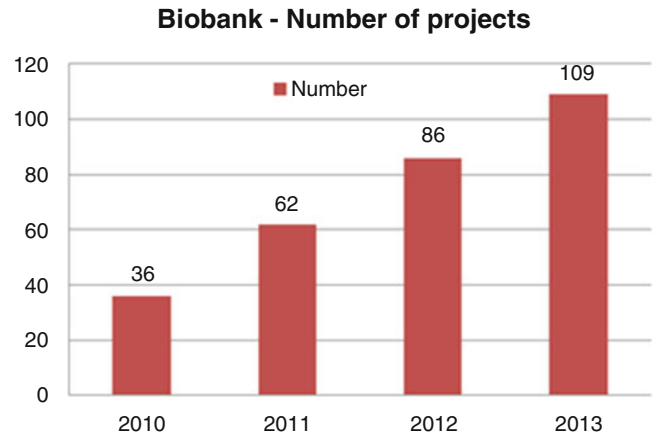

Fig. 9.4 Number of research projects that required access to samples stored at the A. C. Camargo Biobank in the last 4 years

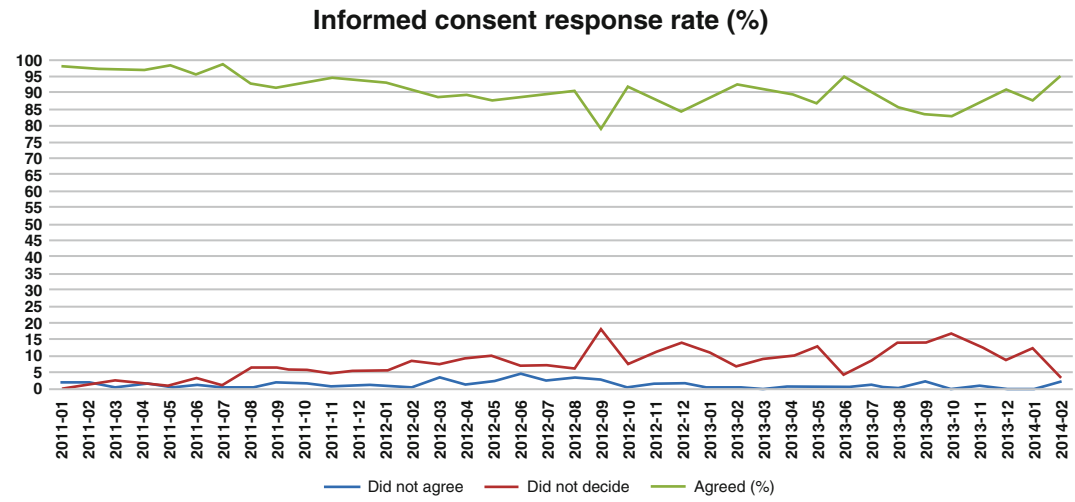

Fig. 9.5 Evolution in the rates of denial, accepting and blank (undecided) informed consents between January 2011 and February 2013. The decrease in the rate of accepting informed consents is due to an increase in rate of undecided donors

recontacted to clarify the reasons for not having reached a decision regarding biobanking for future research use. This very process would be time consuming, expensive and require ethical approval to be carried out. Since post-operative consenting is currently not accepted under Brazilian regulations, the rate of missed collections in some months exceeded 15 %. Until there is solid information about the Brazilian public's perception on biobanking or adopting different modalities of informed consent, our institution, as well as others involved in biobanking, will have to revise the strategies for obtaining consent in order to reduce the rate of undecided responses. The best strategy for one institution may not be considered suitable for another. For example, the informed consent process for banking at the A. C. Camargo Cancer Center is carried out by the hospital doctors, whose primary concern is patient care. The cost of employing dedicated staff for consent taking or diverting other professionals (for example, nurses or health care assistants/nursing auxiliaries) to fulfill this need would have to be weighed against other institutional priorities [19].

It is also expected that the challenge of implementing and maintaining an effective informed consent process will be more difficult after the Brazilian new regulatory landmark, that granted donors the option of conceding either (i) a general consent authorizing use of the biological materials in any future research project, or (ii) a limited consent requiring reauthorization for future projects [13]. Donors can also specify whether or not they wish to be re-contacted should any relevant personal health information be derived from use of their samples. Not only tumor banks but any institutional biobank (as defined in the Brazilian regulations) will have to track consent decisions, as investigators will have to (i) seek reauthorization for use of samples in their specific projects, and (ii) know which donors will have to be re-contacted to receive any significant information derived from the use of their samples. Although the new regulatory landmark softened consent requirements (since a one time, general-informed consent was not authorized by previous regulations), it remains to be seen if this strategy to foster biobank-based research while balancing individual and collective rights will not introduce a "selection bias" when

investigators require access to samples stored in biobanks.

9.5 Concluding Remarks

Since the beginning of the A. C. Camargo Tumor Bank in 1997, tumor banking has become a hot topic in Brazil and other Latin American countries. Given the challenge that this region will face in the coming years, with cancer cases continuing to soar, it is time for a concerted effort towards creating well-established tumor banks. What is already a challenging activity in developed countries can become even more challenging in a region where some particularities exist. The difficulties in obtaining adequate funding may prevent emerging tumor banks from achieving growth and prosperity. Aside from Brazil, no other country has implemented specific regulations on biobanking for health research. Although it is expected that some countries will introduce their own regulations in the coming years, the lack of regulatory landscape for biobanking will continue to constrain the emerging of new tumor banks. In Brazil, it remains to be seen how effective the new national regulations will be in fostering biobanking activities. We expect that that our views and experience in operating a 17-year old cancer-oriented biobank in Brazil may be helpful for the development of other similar biobanks in the region.

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China Biobanking

10

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Abstract

Biobanks are playing increasingly important roles in clinical and translational research nowadays. China, as a country with the largest population and abundant clinical resources, attaches great importance to the development of biobanks. In recent years, with the increasing support from the Chinese government, biobanks are blooming across the country. This paper provides a detailed overview of China biobanking, which is further divided in the following four parts: (i) general introduction of the number, category and distribution of current biobanks; (ii) summarization of the current development status, and issues that Chinese biobanks are faced with; (iii) international cooperation between China and the global biobanking community; (iv) prospect of the modern twenty-first century Chinese biobanks, which would achieve standardized operation, systematic specimen management, and extensive collaboration, and thus provide support for the robust research discoveries and personalized medicine etc.

Keywords

China biobanking • Specimen • Standardization • Management • Cooperation

10.1 Introduction

The term of "biobank" used to defined as "an organized collection of human biological material and associated information stored for one or more research purposes" [1, 2]. It first appeared in the scientific literature in 1996 and for the next five years was used mainly to describe human population-based biobanks [3], the material they collected is mainly human tissues. Nowadays collections of plant, animal, microbe, and other

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nonhuman materials can also be classified as biobank [1]. In this paper, we will focus on human biobanks.

Many developed countries have established standardized biobanks, for example UK biobank, JANUS Serum Bank in Norway, Integrated BioBank of Luxembourg, etc. Meanwhile, for better use and exchange of resources and address kinds of issues related to biobanking, many biobank communities and network platforms are arisen, which includes the International Society of biological and Environmental Repositories (ISBER), the Office of Biorepository and biospecimen Research (OBBR) built by National Cancer Institute (NCI) in USA, the Biobanking and Bimolecular Resources Research Infrastructure (BBMRI) in Europe, etc. [4]. The high standard biobanks could powerful support the development of scientific research, biological medicine and clinical study [5].

With the rapid development of biobanks around the world, Chinese biobanks are blooming and make great progress these years. Since early in 1970s, some hospitals have already started collecting specimens and established their own biobanks [6]. In 1994, Chinese academy of science established the Immortalize Cell Bank of Different Chinese Ethnic Group aiming to reserve different Chinese ethnic groups' genomes [7]. In recent years, with increased attention from government, academia, and society, more and more biobanks are built up in China. Here, we give an overview of the current biobanks in China, summarized the development status, kinds of issues Chinese biobanks facing now and international cooperation and prospect about what a modern twenty-first century biobank will be developed in the future.

10.2 Chinese Biobanks

Most of the Chinese biobanks established in last 10 years [5]. In recent years, with the increasing investment and emphasis on translational medicine research by government and academia, the biobanks in China developed very quickly. Here we summarized some China biobanks based on information we get from forums, seminars and published papers (Table 10.1). Depending on purpose and design, human biobanks generally fall into two categories; the disease-oriented biobanks and population-based biobanks [1]. What's more, since China has a relatively stable population and a variety of geography features, Chinese biobanks have some characteristics of their own.

10.2.1 Disease-Oriented Biobanks

Most of the disease-oriented biobanks in China are established by hospitals, and closely connected with the hospitals' specialties and research projects. Large scale biobanks are gathered in big cities where the medical systems are better.

In 2008, supported by Shanghai Science and Technology Commission, Shanghai Development and Reform Commission and Shanghai Ministry of Health, Shanghai Clinical Research Center (SCRC) was appointed as the third-party coordinator to cooperate with eight university hospitals in the initial stage to establish the prototype of the Shanghai Biobank Network (SBN) [6]. They devoted to the improvement of biobank standards and the development of translational medicine. The network has 18 allied units and most of allied members are specialized hospitals. By the year of 2011, they've already stored over 100,000 specimens in the network hospitals. Fudan University Shanghai Cancer Center is one of the allied units of SCRC, and built a biobank in 2006 collecting peripheral blood, tissue as well as various body fluids, the number of specimens is about 166,000 till now. The Sixth People's Hospital Affiliated to Shanghai Jiao Tong University is also a member of SCRC focusing on metabolic diseases, and has collected a large amount of samples from about 10,000 diabetes and osteoporosis patients now.

Beijing biobank of Clinical Resources (BBCR), sponsored by Beijing Municipal Science and Technology Commission, is established in 2009 with the mission of constructing a common platform based on rich patient's resources in Beijing to support Beijing biomedical industry. Capital Medical University is the primary institute to found Project Management

Table 10.1 Chinese biobanks

	Hospital/Institute	Location	Founding time	Scope	Specimen type	Website
Northern China	Beijing Cancer Hospital	Beijing	1996	Disease-oriented	Tumor tissue, blood	www.bjcancer.org
	Tianjin Medical University Cancer Hospital	Tianjing	2003	Disease-oriented	Tissue, blood	www.tjmuch.com
	Tianjin Huanhu Hospital	Tianjing	2011	Disease-oriented	Tissue, blood and cerebropinal fluid	www.tnsi.org
	Capital Medical University affiliated Beijing Anzhen Hospital	Beijing	2012	Disease-oriented	N/A	www.anzhen.org
	Hebei Ci County Cancer Hospital	Cixian	2012	Disease-oriented	Tissue, blood	N/A
	The PLA General Hospital	Beijing	N/A	Disease-oriented	Blood, DNA, urine	www.301hospital.com.cn
	YouAn Hospital affiliated to Beijing Capital Medical University	Beijing	N/A	Disease-oriented	Tissue, blood	www.bjyah.com
	Peking Union Medical College Hospital	Beijing	N/A	Disease-oriented	Blood, frozen tissues, paraffin-embedded tissue, urine, pathological section, pancreatic juice, cerebrospinal fluid et al.	www.pumch.cn
	The China Marrow Donor Program	Beijing	1992	Population-based	N/A	www.cmdp.com.cn
	The Immortalize Cell Bank of Different Chinese Ethnic Groups	Beijing	1994	Population-based	Cell line, DNA	N/A
	China Kadoorie Biobank	Beijing	2003	Population-based	Blood	www.ckbiobank.org
Central China	The First Affiliated Hospital of Zhengzhou University	Zhengzhou	1995	Disease-oriented	N/A	www2.zzu.edu.cn/cancer
	Eighth Hospital of Wuhan City	Wuhan	2007	Disease-oriented	Tissue, blood, plasma, serum, biological fluid, DNA, RNA and proteins	www.wh8yy.cn
	National Engineering Research Center of Human Stem Cell	Changsha	2012	Disease-oriented	N/A	www.nercsc.com/cn
	Chinese Human Sperm Bank	Changsha	1981	Population-based	Sperm	www.hunan.cnspermbank.com

(continued)

Table 10.1 (continued)

	Hospital/Institute	Location	Founding time	Scope	Specimen type	Website
Easten China	Nanjing General Hospital of Nanjing Military Command	Nanjing	1994	Disease-oriented	Serum, plasma, blood DNA, urine, Kidney frozen section, paraffin-embedded tissue	www.njzy666.com
	Tong ji Hospital	Shanghai	1999	Disease-oriented	Tissue, blood, DNA, cell	www.tongjihospital.com
	Wuxi No. 4 People's Hospital Affiliated to Suzhou University	Wuxi	1999	Disease-oriented	Tissue, RNA, serum, plasma, lymphocyte, stem cell and cell line	www.wuxihospital.com
	National biochip engineering research center of Shanghai	Shanghai	2002	Disease-oriented	Tissue, blood	www.shbiochip.com
	Taizhou Hospital	Taizhou	2004	Disease-oriented	Fresh frozen tissue, paraffin-embedded tissue, blood, serum, plasma and cerebrospinal fluid	www.tzhospital.com
	Zhejiang Cancer Hospital	Hangzhou	2007	Disease-oriented	Tissue, tumor adjacent tissue, benign tumor, serum, plasma and white blood cell layer	www.zchospital.com
	Eastern Hepatobiliary Surgery Hospital, the Second Military Medical University of Chinese PLA	Shanghai	2009	Disease-oriented	Tissue, paraffin-embedded tissue, blood, urine, DNA, RNA	www.ehbh.cn
	Jiangsu Province Hospital	Nanjing	2010	Disease-oriented	Tissue, blood	www.jsph.net
	Wuxi Mental Health Center	Wuxi	2010	Disease-oriented	Blood	www.wuximhc.com
	Xin Hua hospital Affiliated to Shanghai Jiaotong University School of Medicine	Shanghai	2011	Disease-oriented	Tissue, blood, blood slides	www.xinhuamed.com.cn
	Qidong liver cancer institute	Shanghai	2011	Disease-oriented	Serum, plasma, white blood cell, tumor tissue, paraffin-embedded tissue, urine	www.qdlci.ac.cn
	Children's Hospital of Shanghai	Shanghai	2012	Disease-oriented	Blood, blood slides etc.	www.shchildren.com.cn
	The Six People's Hospital Affiliated to Shanghai Jiao Tong University	Shanghai	N/A	Disease-oriented	Serum, urine, DNA	www.6thhosp.com
	Fudan University Shanghai Cancer Center	Shanghai	N/A	Disease-oriented	Serum, blood clot, plasma, Mononuclear cells, tumor tissue and normal tissue, urine, ascitic fluid, hydrothorax	www.shca.org.cn
	Shuzhou University	Suzhou	N/A	Disease-oriented	Tissue	www.suda.edu.cn
	The Children's Hospital Affiliated to Zhejiang University School of Medicine	Hangzhou	N/A	Disease-oriented	Blood, tissue, body fluid, cell	www.zjuch.cn
	The Third People's Hospital of Nanchang	Nanchang	N/A	Disease-oriented	Blood, tissue	www.ncsyy.com
	Taizhou (Fudan University) Institute of Health Sciences	Taizhou	2007	Population-based	DNA	www.fdcmc.com
	The Fifth People's Hospital of Shanghai, Fudan University	Shanghai	2010	Population-based	Blood, urine	www.5thhospital.com
	Ren Ji Hospital (Shanghai Jiao Tong University)	Shanghai	N/A	Population-based	Serum, plasma, DNA, urine, saliva and endocrine tumor tissue	www.renji.com

Southern China	Sun Yat-sen University Cancer Center	Guangzhou	2001	Disease-oriented	Blood, serum, plasma, blood cells, bone marrow, cell, protein, DNA, RNA and paraffin section	www.sysucc.org.cn
	Guangdong Lung Cancer Institute	Guangzhou	2003	Disease-oriented	Tissues, tumor adjacent tissue, adjacent, non-cancerous tissue, blood, hydrothorax	www.lungcancer.dxyer.cn
	The Tumor Hospital of Guangxi Zhuang Autonomous Region	Guangxi	2006	Disease-oriented	Tissues, tumor adjacent tissue, adjacent, non-cancerous tissue, serum, plasma and lymphocyte	www.gxzlyy.com
	The Sixth Affiliated Hospital of Sun Yat-sen University	Guangzhou	2007	Disease-oriented	Tissues, serum, plasma, blood cells, blood and faeces	www.zs6y.com
	Shenzhen PKU-HKUST Medical Center	Shenzhen	2009	Disease-oriented	N/A	www.sphmc.org
	The Second People's Hospital of Shenzhen	Shenzhen	2011	Disease-oriented	Tissue, blood, bone marrow, cerebrospinal fluid, saliva, urine, faeces	www.szrch.com
	Guangdong Women and Children Medical Center	Guangzhou	2011	Disease-oriented	peripheral blood, umbilical cord blood, placenta, DNA	www.e3861.com
	CNGB	Shenzhen	2011	Disease-oriented	Blood, urine, faeces, saliva, tissue, cell etc.	www.cngb.org
	The Guangzhou Biobank Cohort Study	Guangzhou	2003	Population-based	Plasma and leukocytes	N/A
	Born in Guangzhou Cohort Study	Guangzhou	2010	Population-based	Blood	N/A
Western China	West China Hospital	Chengdu	2009	Disease-oriented	Tissues, adjacent tissues and blood, plasma, white blood cell, oral mucosa	www.cd120.com
Northwest China	The First Affiliated of Hospital Xinjiang Medical University	Urumchi	2004	Disease-oriented	Tissue, blood	www.xydyfy.cn
	Cancer Hospital Affiliated to Xingjiang Medical University	Urumchi	N/A	Disease-oriented	Tissue, blood	www.xjzlyy.com
	The Key Laboratory of Xinjiang Endemic and Ethnic Disease	Urumchi	2004	Population-based	Blood	N/A

N/A for not available

Committee. The BBCR consists of 15 members and these members work as in one team to construct parallel, separately 16 sub-biobanks, involving cerebrovascular diseases, HBV, HIV/AIDS, emerging infectious diseases, psychiatric disorders, cardiovascular diseases, tuberculosis, cervical cancer, breast cancer, CKD, diabetes and degenerative orthopedics diseases. Beijing Cancer Hospital, the PLA General Hospital and the YouAn Hospital are member hospitals of BBCR too. Beijing Cancer Hospital established a clinical tumor biobank in 1996 [8]. It has collected about 500,000 specimens up to now involving blood, human tumor tissue and tumor adjacent tissues. The hospital aims at studying of various kinds of cancers related to gastric, esophageal, colorectal, breast, liver etc. The PLA General Hospital located in Beijing is the largest military general hospital in China. The hospital includes 6,000 beds and has about 10,000 outpatients a day. By the end of 2013, they have collected at least 180,000 samples including blood, DNA, urine from more than 7,700 cases. It is also planning to build a kidney disease biological resource center with the help of Beijing Municipal Science and Technology Commission. YouAn Hospital affiliated to Capital Medical University is one of the largest facilities in China and participates in NIH/AIDS trials. The hospital has 1,000 beds, average 2,000 outpatients a day and specialized in the technical of artificial liver support, liver transplantation, AIDS treatment and care, Chinese medicine treatment of liver disease, etc. Over 500,000 specimens have been collected from more than 6,000 patients.

In Guangzhou, Sun Yat-sen University Cancer Center and the State Key Laboratory of Oncology established a tumor biobank in 2001 which has been one of the largest biobank in China now. It collects specimens from tumor patients and health people; the type of specimens includes blood, serum, plasma, blood cell, bone marrow cells, proteins, DNA, RNA, paraffin sections. The latest number of specimens is 1 million. The biobank of Guangdong Lung Cancer Institute started running in 2003 and focuses on the study of lung cancer. 92,000 specimens have been collected covering tumor tissues, tumor adjacent tissues, non-cancerous tissues, blood and hydrothorax tissue, etc. The Sixth Affiliated Hospital of Sun Yat-sen University is specialist in gastrointestinal diseases, which started collecting tissue, blood and faeces samples since 2006.

In addition, the Tianjin Medical University Cancer Institute and Hospital is treated as the birthplace of China Oncology. The hospital has 2,000 beds and about 80,000 outpatients a year. Their tissue bank had already collected 35,000 blood samples and 36,000 tissues by the year of 2013. Zhejiang Cancer Hospital's tumor biobank is established in 2007 [9]. It is a fully functioned biobank guided by Yale University and have stored over 140,000 specimens now. The Specimens Bank of Xingjiang Key Diseases set up in 2004. They focus on specimens collection from high incidence diseases in Xinjiang. 50,000 blood and tissues samples have been maintained now.

10.2.2 Population-Based Biobanks

The China Marrow Donor Program (CMDP) was established in 1992 but start running in 2002. It becomes a member of Bone Marrow Donor Worldwide (BMDW) in 2012, and by the end of the year the number of recruited potential Hematopoietic Stem Cell (HSC) donors of CMDP reached 1.65 million.

The China Kadoorie Biobank (CKB) is set up in 2003 and aims to investigate the main genetic and environmental causes of common chronic diseases in the Chinese population. During 2004–2008, CKB recruited 510,000 adults from ten geographically defined regions of China; their extensive data was collected and blood specimens were stored for future study. Every few years, they conduct periodic re-surveys in about 25,000 surviving participants.

Guangzhou Occupational Diseases Prevention and Treatment Centre collaborate with The Universities of Birmingham and Hong Kong started a research project named the Guangzhou Biobank Cohort Study (GBCS) in 2003. The project focuses on older people aged at least 50 years lives in megacity with the

population over ten million. So far, they have recruited 30,000 participants. The aim of the study is to examine the effects of genetic and environmental influences on health and chronic disease development [10].

In 2004 the key Laboratory of Xinjiang Endemic and Ethnic Disease set up a biobank for ethnic diseases research in Xinjiang. In 2007, Fudan University established a biobank in Taizhou based on a population health-tracking research project, which collects 159,100,000 DNA specimens until now. Guangzhou Women and Children's Medical Center started a cohort study named the Born in Guangzhou Cohort Study from 2010 with the objectives to find the main factors affecting children and women health. The project planned to follow 1,000,000 pregnant women and their children for 20 years. Moreover, in the same year, the Fifth Hospital of Shanghai built the Institutional Specimen biobank, they have collected over 100,000 blood and urine specimens for chronic diseases research.

Germ cells and kinds of cell lines are preserved by some Chinese biobankers for medical applications or scientific research. China first sperm bank, founded in Changsha in 1981, has in all stored 250,000 specimens of semen and produced more than 40,000 tube babies by the year of 2013 [11]. The Immortalize Cell Bank of Chinese Ethnic Groups established in 1994 is the largest immortalized cell lines bank in China. 3,982 immortalized cell lines and 7,210 DNA samples from 70 ethnic groups were collected until 2008 [7].

10.2.3 Characteristic China Biobanks

China is a vast county with vast territory, numerous nationalities and abundant genetic resources, most of the Chinese people live in a compact community, and the families have a relatively simple genetic background, though the condition have been diluted by the development of economy, transportation, and the change of lifestyle partly [12]. So the establishment of some biobanks has regional characteristics.

Esophageal cancer (EC) remains a leading cause of cancer-related deaths in Linzhou (formerly Linxian) and Huixian of Henan province (Central China), which has been well recognized as the highest incidence area for EC in the world attract extensive attention [13, 14]. The First Affiliated Hospital of Zhengzhou University established the Henan Key Laboratory for Esophageal Cancer in 1995. The laboratory established a Esophageal biobank and for the past 20 years, trying to explore the interaction of environmental and hereditary factors on human esophageal by combining follow-up study with molecular biological technique. The Ci county in Hebei Province (North China) also has a very high rates of oesophageal cancer, and the biobank related to esophaqus cancer was set up in 2012, and collects tumor and adjacent tissues, plasma, white blood cell, normal tissues and corresponding personal information, aiming at building a cohort study based on high risk patients by endoscope screening [15].

Thalassemia is one common inherited disease in Guangdong and Guangxi provinces (South China), Guangdong Women and Children Hospital take this advantage to establish a biobank and collect peripheral blood, umbilical cord blood, placenta, DNA from newborns for thalassemia study and other birth defects since 2011 [16].

Qidong (Eastern China) has a high incidence of liver cancer, and Qidong Liver Cancer Institute established a cancer biobank in 2011, the specimens they collect include serum, plasma, white blood cell, tumor tissue, urine [17]. Guangdong province is also treated as a high incidence area for liver cancer. The Zhujiang hospital affiliated to Southern Medical University set up a tumor tissue bank and a data bank in 2010, they collect specimens of the tissue fragments, nucleic acids, serum, plasma, lymphocyte and stem cells, etc.

10.3 The Status Quo of Chinese Biobanks

As a large developing country, China is rich in clinical specimens and bio-resources, the wide variety of specimen types and big population base in China make it easier and cost lower for extensive specimen obtainment compared to other countries [18]. Like other countries, Chinese biobanks also face diverse problems and challenges at the primary stage.

10.3.1 Biobank Scale

The biobank scale varies greatly from simply fulfill periodical studies to large scale research projects. For small biobanks, some of them like medical organizations collect and storage specific specimens related to the characteristic of the hospital or a certain clinical department spontaneously without project design or a long-term plan, and some biobanks are only established to match the research tasks within a certain period of time. There are some well-designed, standardized and organized larger biobanks with professional facilities, dedicated personnel and management organization etc.

10.3.2 Specimen and Related Data Sharing

Many biobanks are operated independently with less communication mutually in China. Since no official unified-standard implemented, the process of specimen and related data collection, handling, and storage follows distinct guidelines among biobanks, which leads to uneven specimen quality, and limits the specimen sharing in some degree [19]. And since no universal network connection applied which allows implementing consistency among Chinese biobanks, the data including clinical information, specimen information, derived data and follow-up information can not be effectively integrated, shared and utilized among biobanks. It is necessary to properly integrate specimen and related comprehensive information, and establish sharing platform among biobanks for efficient and rational application of valuable bio-resources. And increasing biobanks are seeking cooperation based on the specimens, and also attempt to share construction and management experience with each other those years through multiple ways like founding biobank alliance and launching academic exchanges etc.

10.3.3 Biobank Staff

Staff allocation is a critical element for biobanking, and is in charge of the biobank operation or responsible for the management of specimen similar to surgeon, pathologist, and biobank staff and others who would participate in specimen collection, evaluation, transportation and handling, etc. In order to ensure the specimen quality, involved individual also must be trained strictly to accurately and effectively execute their tasks. Currently, lots of the biobank employees are part-time and composed of school or hospital staff. The biobanks are relative shortage of the qualified and specialized technician and management personnel in China. Although there still lack of system technical training and qualification authentication for the staff in China [20], some organizations are carrying out series of lectures and classes to strengthen practitioners' knowledge exchange by inviting specialists in biobanking field from both domestic and overseas.

It is not easy for ordinary people without any medical background to understand the objective and the research contents of the establishment of biobanks, and certain privacy risks exist when personal data applied in biobanking, so how to reassure the stakeholder and obtain more support and trust is a very important and urgent issue in China biobanking. Now, many biobanks construct the website or publish on newspapers to spread biobank-related knowledge and to report the research progress, which provide clear, understandable and acceptable ways for the participants. And transparent supervisory systems and regulations are necessary for protecting the legitimate rights of the stakeholder.

10.3.4 Laws and Regulations

So far, there is no specialized law or oversight mechanism against biobank standardized management in China, although some regulations related to human genetic resources were enacted like *Interim Review Procedures for Human Biomedical Ethics*, *Interim Measures for the Administration of Human Genetic Resources*, but both of them are departmental regulations with low legislative level and weak enforcement, which are imperfect and lack of detailed rules, and not convenient for execution. And a unified standardization system involves every aspect of biobanking is appealed to be established by Chinese government covering terms, data format, standard operating procedures, quality control, specimen sharing, ethics issues, privacy and safety, etc. [21].

10.3.5 Funding

It is costly to build and operate a biobank which must be supported by funding and high technology. Most of the biobanks in China are non-profit and funded by government or research grants, fund shortage is a crucial bottleneck problem for the development of Chinese biobanks. Recent years, Chinese government and relevant authorities enlarge financial allocation to support biobank construction (Table 10.2), like *China's Twelfth Five Year Plan* (*2011–2015*) *for the Development of Biological Technology* which clearly outlined to support the establishment of large scale biobanks. And the commercial operation could be a new approach for biobank sustainable development which needs more exploration in the future.

10.4 Ethical Issues

Ethical review is a considerable issue and plays rather important role in biobanking for strengthening the guidance and supervision of scientific research and protecting the donors' safety, privacy, the right and interest etc. Closer attention related to ethical issues need to be paid in the process of extensively collect, store and utilize human genetic resources and relevant information and data, involving the consent procedures, the privacy and confidentiality, intellectual property and conflict of interest etc. Though there is no national specialized and detailed ethical principle and review regulation formulated in China, ethical issues have attracted more and more concern for the public especially biobankers in China. Different biobanks established Ethics committee, drafted biobank ethical management guidelines of their own in order to properly handle ethical problems. How to enhance the ethical construction is one of the major subjects and key points in sorts of meetings and symposium. And the particular problems occurred with the development of biobanks triggered widely discussion and exploration such as how to enable the 'vulnerable groups' to get better protection, and how to deal with the public acceptance, retribution, security etc. issues related to human genetic clinical research.

Last several years, in order to achieve biobank effective operation, Chinese biobankers conduct different attempts and bring some innovative patterns for biobanking like China National Genebank (CNGB), which consists of biological bank, information database (clinical information and omics information) and collaboration alliance network together, aims to construct comprehensively bioresource and information network and platform. CNGB collects and stores traceable specimens and related data, devotes continuously in developing standard specification, and also provide various services such as biobanking solutions, skill training for personnel, specimen storage service for other organizations, which creates a new biobanking model through bio-resource to scientific research and industry.

10.5 International Cooperation

With the establishment of numerous biobanks in China over the past two decades, much progress has been made in Chinese biobanking in terms of infrastructure construction, quality management

Table 10.2 Policies for Chinese biobanks

Policy	Formulation Department	Time	Website
The National Medium- and Long-Term Program for Science & Technology Development (2006–2020)	The State Council of PRC	2005	www.gov.cn/gongbao/content/2006/content_240244.htm
Several Policies to Promote and Speed Up the Development of Bioindustry	General Office of the State Council	2009	www.gov.cn/zwgk/2009-06/05/content_1332777.htm
Decision of the State Council on Accelerating the Fostering and Development of Strategic Emerging Industries	The State Council of PRC	2010	www.gov.cn/zwgk/2010-10/18/content_1724848.htm
The national "Twelfth Five-Year" development plan of science and technology	Ministry of Science and Technology of PRC	2011	www.gov.cn/gzdt/2011-07/13/content_1905915.htm
The national "Twelfth Five-Year" development plan of biotechnology	Ministry of Science and Technology of PRC	2011	www.most.gov.cn/fggw/zfwj/zfwj2011/201111/t20111128_91115.htm
The Development Plan of Bioindustry	General Office of the State Council	2012	www.gov.cn/zwgk/2013-01/06/content_2305639.htm

etc. Now China is seeking and welcoming more cooperation with international biobanking community in scientific research, commercial partnerships, as well as academic exchanges etc.

10.5.1 Scientific Research

Taking advantage of its abundant genetic resources and advanced sequencing technology, China has taken part in various international projects. Early in 2004, Tianjin Medical University Cancer Institute and Hospital (TMUCIH), has signed with National Foundation for Cancer Research (NFCR) to jointly establish the Tissue Banking Facility (TBF) in Tianjian. Founded to preserve human tumor tissue samples, TBF not only provides valuable materials for research groups of TMUCIH, but also foster academic exchanges between China and America in the field of tumor science. Through years of joint effort, TBF has become the largest tumor tissue bank in China, with a total collection of nearly 34, 000 tumor tissue specimens and 40,000 blood samples by 2012 (http://www.tijmu.edu.cn/s/2/t/250/21/3d/info8509.htm). Another collaborative research project that needs to be mentioned is the recently initiated EpiTwin project, the biggest epigenetics project in the world. The EpiTwin project aims to capture the subtle epigenetic profiles that mark the differences between 5,000 twins on a scale and depth. It is initiated by the famous TwinsUK research group from King's College London in 2010, in collaboration with BGI-Shenzhen (http://www.epitwin.eu/). The EpiTwin project is progressing well, in which some related publications can already be found online.

10.5.2 Commercial Partnerships

Chinese biobanks have established a wide range of partnership with foreign companies. CNGB, for example, has announced to develop a strategic alliance agreement with BioStorage Technologies, Inc. (BST) (http://www.genomics.cn/en/news/show_news?nid=99734). CNGB is China's first national genebank (http://www.nationalgenebank.org/en/index.html), while BST is the premier, global provider of comprehensive sample management solutions (http://www.biostorage.com/). The establishment of a strategic alliance between these two partners will definitely bring a brand new face to the development of bioscience industry in China. Thermo Fisher Scientific Inc., the world leader in serving science, has maintained a long-term and stable partnership in providing integrated biobanking solutions for the Tianjin Cord Blood Stem Cell Bank. Other commercial partners for Chinese biobanks include: Hamilton Robotics (Switzerland), Tecan (Switzerland), Eppendorf (Germany), QIAGEN (Germany), Cryo Bio System (France), Beckman Coulter (America), CryoXtract (America), AIR PRODUCT (America), PerkinElmer (America), Affymetrix (America), ESCO (Singapore), Avan Tech (Canada), and Panasonic (Japan). (http://bbc-mba2014.biobank.org.cn/en/). These companies are either providing sample storage facilities or technologies, automatic laboratory stations for sample handling, or technical solutions for China biobanks. They are putting more emphasis on the Chinese biobanking market in recent years.

10.5.3 Academic Exchanges

At the mention of biobanking conferences, one cannot skip the annual meeting of the International Society for Biological and Environmental Repositories (ISBER) (http://www.isber.org/). ISBER is the largest forum which connects repositories globally through best practices. ISBER consists of 9 working groups, in which the new Trans-omics Working Group was initiated by CNGB for sharing valuable experiences related to omics specimen study. The ISBER meeting is held annually and is attracting more and more Chinese biobankers. Over the past 5 years, the number of Chinese attendee increases from 2 to 64, making up the largest percentage of attendance (nearly 10 %) among the 34 countries in 2014. On Jan. 1st, 2014, Professor Jim Vaught, the president-elect of ISBER in 2015, was

appointed as the Senior Advisor to the President of the SCRC (http://www.scrcnet.org/enews.asp?id=207). He is mainly responsible for the consultation work to SCRC for Shanghai biobanking project and biobank development in China.. The journal of Biopreservation and Biobanking is becoming more and more influential recently, in which some research paper from Chinese biobanks have already been published. Another one is the meeting of ISO/TC 276 Biotechnology. ISO/TC 276 is set up by the International Standards Oganization (ISO) in 2013, during which China was included as one of the 20 participating countries. ISO/TC technology deals with the standardization of terms and definitions, bioresource, biobanking etc.(http://www.iso.org/iso/home/standards_development). Chinese delegation attended the annual meeting of ISO/TC276 in May this year (http://www.genomics.cn/news/show_news?nid=100009), and the meeting will be held in Shenzhen, China next year. Other renowned international conferences include the Sino-American Symposium on Clinical and Translational Medicine (http://www.chinacts.org/), US-China Workshop on Developing Common Standards on Biorepositories and Biospecimen Research (http://www.tjmuch.com/system/2011/10/19/010090658.shtml), the Annual Biobank China (http://www.scrcnet.org/biobank2013/eindex.asp), China Biobank Standardization and Application Seminar (CBSAS) (http://bbcmba2014.biobank.org.cn/en/) etc. These regularly held conferences have attracted biobanking specialists both from China and many other countries to exchange ideas, share best practices, learn about new progresses, and addressing issues that are related to biobanking.

10.6 Prospects for Twenty-First Century Chinese Biobanks

10.6.1 Standardization of the Procedures

The basic procedures and function for biobank are to collect, process, store, disseminate and dispose specimens and related data, all those steps are essential for specimen quality and could further affect the application like diagnostic and translational medicine research [22, 23]. In order to ensure the high quality of specimens, and facilitate specimens and information exchange among different biobanks, lots of organizations are committed to developing and sharing 'best practices' or SOPs (Standard Operation Procedure) with the public [24–26]. The most well known 'best practices' include *Best Practices for Repositories* developed by ISBER [26], and *Best Practices for Biospecimen Resources* published by NCI (http://biospecimens.cancer.gov/practices/default.asp).

With the purpose of improving specimen quality and effective utilization, and addressing the gap with well-developed biobanks, plenty of the works have done by Chinese government and biobankers. Standardization Administration of the People's Republic of China enacted China's Twelfth Five Year Plan for the Standardization Development (http://www.sac.gov.cn/sbgs/zcxwj/bzhgl/201312/t20131224_149252.htm) to encourage the establishment of biological standard system. There are no national standards or industry standards for biobanking in China, released as local standards for the first time, three documents named the Regulation on Animal Germplasm Bank Construction and Management (SZDB/Z 89–2014, http://www.szaic.gov.cn/xxgk/qt/ztlm/szdfbz/tzgg/201401/t20140121_2306813.htm), Regulation on Human Biobank Construction and Management (SZDB/Z 91–2014, http://www.szaic.gov.cn/xxgk/qt/ztlm/szdfbz/tzgg/201401/t20140126_2308869.htm), Regulation on Bioinformation Bank Construction and Management (SZDB/Z 92–2014, http://www.szaic.gov.cn/xxgk/qt/ztlm/szdfbz/tzgg/201401/t20140126_2308876.htm) were formally implemented on February 1, 2014 in Shenzhen, the three standards bring guiding role and provide the foundation for the collection, processing and storage animal germplasms, human resources, and bioinformation etc. China Medical Biotech Association Biobank Branch published *Criterion for Biobanking* which organized and was written by specialists from different fields covering clinic, pathology, legal, and biobanks, and aims to standardize the operation procedures. Two books related to best practices for

biobanking were published; Capacity Development and Best Practices for biobanks, and Best Practices for biobanking, both of them provided guidelines for biobank construction covering the informed consent issues, the standards of specimen collection, processing, transportation, storage, quality control etc. All of these initiatives indicate that China's biobanks are moving toward standardization, and standardized processes and regulations allow biobanks to rationally and effectively use high-quality specimens, meanwhile meet the requirements of translational research.

10.6.2 Sample Management

It is equally important to record, process, storage and protect well-annotated data with high quality specimens [27, 28]. The data correlated with specimen increases explosively nowadays, not only because the larger number of the basic specimens information, but also because the development of high-throughput technologies which accumulate tons of original data for further analysis and application, including the information of genome, transcriptome, proteome, metabolome, and epigenome etc. [29–31]. How to deal with and protect those big data are grand challenges for biobanking. Biobank informational management system is necessary to rapidly and accurately track the specimen life cycle and store related data.

RURO focus on software technologies and modern hardware support tools to process execution difficulty, and integrate technologies into all its solutions especially automating a lab work environment (http://ruro.com/). OpenSpecimen (formerly known as caTissue Plus) is a biobank/biospecimen management software with highly configurable and customizable, which streamlines management of biospecimens across collection, consent, quality control, request and distribution (http://www.catissueplus.org/). In China, different organizations developed kinds of Sample Management System, including Hair (http://www.bio-equip.com/showarticle.asp?id=453069068), CapitalBio Corporation (http://blog.sina.com.cn/s/blog_66562cd50100ifdi.html), Avantech (http://www.avantech.cn/Cryopreservation.asp?ID=56) and China National Genebank (http://cngb.org/ability.jhtml) etc. Here we list three sample management systems developed by different organizations (Table 10.3). All of those professional specimen management systems facilitate the integrated management of specimen information, and improve the efficiency of the operations containing the register, retrieval, screen of the specimen and related information. In the future, different management system could progress to unified network which could not only run independently, but also link with each other, and be convenient for the specimen and data communication and sharing among biobanks.

10.6.3 The Cooperation and Communication Among Biobanks

Large scale and high quality of specimen and relevant data are the foundations of high level scientific research achievements, so it is necessary and helpful to establish the platform or network that promotes the widest cooperation and communi-

Table 10.3 Sample management systems

Function parameter	Virtual appliance operation	Web-browser access	Custom property	Humiture supervision	Sample manifest query	Universal interface	On/off-line behavior monitoring
FreezerPro	—	√	√	—	√	—	√
Avantech	√	—	√	—	—	√	—
CNGB	√	√	√	√	√	√	√

cation among biobanks [32, 33]. The Biobanking and Biomolecular Resources Research Infrastructure (BBMRI, http://bbmri.eu/), Canadian Tumor Repository Network (CTRnet, http://www.ctrnet.ca/), European Primate Network (EUPRIM-Net, http://www.euprim-net.eu/), Biobank Ireland Trust (BIT) [34] etc. are world-renowned networks for facilitating collaborations among biobanks and researchers.

In China, the biobanks cooperate in different way, for example region-based or specific disease-driven. The BBCR and SBN are both consist of local hospitals or research institutions to fully integrate and utilize the large scale resources, and improve and accelerate the progress of translational research. The project of National Birth Defects Biobank was launched by CNGB in Shenzhen at end of 2012, in collaboration with China Birth Defect Monitoring Center. (http://www.genomics.cn/news/show_news?nid=99517). The clinical specimens and information of high incidence congenital disorders, monogenetic diseases and unknown pregnancy abnormities are collected in China. In the preliminary phase, 100,000 copies of specimens are planned to be collected, and in following 3 years, this project shall be applied in 100,000 families around 100 cities in China, and a birth defect database with complete information will be built for long term regular visits, finally, a birth cohort network will be formed for information monitoring and sharing. This project could provide essential support for research in genetic and environmental factors affecting congenital disorders, as well as improve technical development for early screening, diagnosis, treatment and recovery of congenital disorders.

E-BioBank information sharing platform, a virtual biobank established by CNGB this year, aims to integrate and promote application of bioresources from domestic and international biobnaks. E-biobank consists of four modules: Toolbox for sharing kinds of knowledge related to biobanking, Specimens Locator for retrieval, orientation and arrangement of integrated resources, Project Resources for providing potential cooperation opportunities for research projects, and Biobank Catalogue for demonstrating the distribution of biobanks and bio-resources in China. E-Biobank will facilitate the sharing of bio-resources and data among investigators, and propel collaboration and application internationally, as well as improve efficient communication and exchange in the scientific community.

Sufficient amount of specimens and derived data are critical for the sensitive and accurate digital healthcare management, clinical test, prevention and treatment of diseases and translational medicine research. Fully consider the differences of population, expand exchange and communication, and integrate all kinds of specimens would be powerful strategies for China to attain credible research findings, precise diagnosis and targeted therapies.

10.6.4 Developing Trends for Specimen Conservation

Researches are become increasingly extensive and in-depth nowadays, which supported by corresponding wider range of specimens and more advanced technologies.

Firstly, the specimen types collected and stored by biobanks are growing. In addition to the regular specimens such as blood, tissue, urine etc., hairs, faeces, saliva, umbilical cord, ascites, stem cells and somatic cell are preserved and utilized by some biobanks in China. In order to take full advantage of genetic materials, both human and non-human resources are collected and studied. For instance, CNGB extensively collects various non-human specimens like living cells, germplasm resources of animals and plants, microbial strains, marine organisms, endangered species, etc. to protect the diversity of bioresources in China, which could be applied for animal and plant molecular breeding, functional gene development, renewable energy exploitation, or conservation of biodiversity.

Secondly, novel technologies provide new insight for specimen preservation. Long fragment read technology allows genome sequencing and haplotyping by using about 100 pg DNA (10–20 human cells), which means trace amount DNA could meet the requirement of sequencing, and

storage of small amount of specimen is enough for DNA sequencing in the future. Maybe it can achieve proteins and metabolites analysis using trace amount of specimens with technological progress, which will contribute to significant cost savings. And different products or reagents are applied for specimen preservation at room temperature for short- or long-term, like RNAlater for protection tissue RNA for 1 week at 25 °C, GenTegra (IntegenX), RNAstable (Biomatrica) and RNAshell (Imagene) for preservation DNA/RNA at room temperature for long-term, these new types of products could improve the specimen quality, are convenient for operation, and provides new directions for specimen preservation.

In conclusion, we detailed the current Chinese biobanks and the developing trends, all the issues described in this part could provide a window on China biobanking, and give some reference experience for other biobanks. In the future, there will be more cooperation and communication among biobanks both in China and abroad for effective specimen utilization, and contributing to translational medicine research.

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Establishing an Iso-Compliant Modern Cancer-Biobank in a Developing Country: A Model for International Cooperation

11

Maher A. Sughayer and Lina Souan

Abstract

King Hussein Cancer (KHCC) is a specialized cancer center that treats both adult and pediatric cancer patients from Jordan and the neighboring countries. KHCC is acknowledged as a leader in cancer treatment in the Middle East and its vision is to maintain its leading position in cancer therapy and research. Hence, KHCC embarked on establishing the first ISO compliant cancer biobank (KHCCBIO) in Jordan.

Currently, there are very few biobanks in the Middle East, hence, KHCC wanted to change this situation by establishing an ISO-compliant cancer biobank which would incorporate all current international guidelines and best-in class practices under an approved quality management system for the benefit of researchers in Jordan, its neighboring countries, and throughout the world. The established biobank would follow the highest ethical standards in collecting, processing, storing and distributing high-quality, clinically annotated biospecimens.

The strategy used in establishing KHCCBIO was based on taking advantage of international networking and collaboration. This in essence led to knowledge transfer between well established organizations, institutions and individuals from Europe and Jordan, in existing technological innovation and internationally recognized quality standards. KHCC efforts were facilitated by a grant from the European Union under the seventh frame work program.

Future aims of KHCCBIO are to develop KHCC's research infrastructure, increase its scope and visibility and improve its competitiveness

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throughout the biomedical science arena. Moreover, KHCCBIO is aiming to establish a platform for future knowledge transfer and collaborative research; develop partnerships between European and Middle Eastern organizations.

Keywords
Jordan • KHCCBIO • Cancer • International collaboration • Middle East • Biobank • Europe

11.1 Introduction

In the post-genomics era of biomedical science, biospecimens are assuming an even more prominent role in efforts to identify the key genes, RNAs, proteins, and signaling networks involved in cancer and then use this information to detect cancer at its earliest stages and develop a personalized therapeutic regimen to treat it. Indeed, the very future of personalized medicine depends on the ability of researchers to compare the molecular workings from hundreds of biospecimens and tease out the differences that have diagnostic and therapeutic value. Unfortunately, the vast majority of the millions of biospecimens currently in collections around the world are not suitable for making the type of direct comparisons that modern cancer biology research demands. The reason is simple; no standard protocol has governed how surgeons collect tissue samples, how pathologists prepare those biospecimens, and how tissue banks store their collected biospecimens. Given the exquisite sensitivity of today's analytical techniques, it is sometimes impossible to distinguish between molecular fingerprints related to cancer and that arising from the way a given biospecimen was handled.

Therefore, the importance of establishing a cancer biobank is to achieve the quest of the modern world in the field of cancer research in promoting the principle of personalized medicine. This innovative approach is based on the application of a unique treatment protocol adapted for each patient separately and requires the need for reliable clinical data of the patient. This so-called personalized medicine should be able to reduce the negative effects and conclusively determine the receptiveness of the patient's response to the cancer treatment and prevention of cancer relapse. Personalized medicine works on establishing the best treatment protocols and medical practices according to the needs of each patient, and by referring to the genetic information of each patient, this leads to improved preventive medicine care, and the treatment given to the patient.

Moreover, the purpose of establishing the bank is to take the surplus-cancer tissue collected from patients after filling a consent form and process it and store it according to the highest standards of ISO-accredited quality protocols. Thereafter, this tissue will be used in clinical research studies aiming for personalized medicine without exposing the patient's confidential information. In addition, these high quality samples might also be used in conducting research studies designed to explore the environmental and genetic factors that influence the occurrence and development of cancer and its patterns of spread and linking these changes with the habits of peoples such as the patterns of smoking and dietary habits and lack of practices of sports, as well as changes in other aspects of life which may increase the incidence of cancer disease in the future. The analysis and study of these samples would also support the earlier diagnosis of cancer and gives a clearer picture of the disease stages and predictability of progression that may occur to in the future. Finally, it is envisioned that the cancer biobank will be a gateway to collaborate with other clinical and research centers both locally and globally, in order to improve the health and safety of the patients through receiving the optimal treatment.

Hence, in a pioneering step King Hussein Cancer Center through its networking skills and expertise signed a partnership agreement with its collaborators from Ireland and Switzerland, in an endeavor to establish a cancer biorepository Bank, the first of its kind in Jordan and the region in 2011. This agreement was supported by the seventh framework program of the European Union.

Before we present our experience in establishing the biobank in Jordan let's first have a brief look at the perception of the public for medical research and biobanking in Jordan and the Middle East region.

11.2 Perception for Biomedical Research and Biobanking in Jordan and the Middle East

The participation of the public in biobanking and their positive attitude and support for medical research is extremely important for the success of any biobank in accomplishing its tasks. Therefore it is extremely important to gauge the public opinion and perception before starting a biobank to insure that it will be able to attract sufficient number of volunteers to donate biospecimens. Studies will also be useful to find out factors that would influence the public decision to participate in biobanking and also to find out their concerns in that matter and would help design strategies to address these concerns. It would also give a clear idea regarding policy development and help design these policies and consent types.

The public perception for biobanking varies from country to another and even within the same country among different population groups. In Europe for example according to Gaskal et al. [1] public perception of biobanks is characterized by a striking heterogeneity. While there is a group of North European countries that are rather enthusiastic about the potential of biobank research, the publics of many Central and Southern European countries have substantial reservations when it comes to participating in biobank research, donating tissue, and giving broad consent for research.

In addition more than two thirds of all Europeans said they have never heard of biobanks, and only 17 % answered that they had actively talked or searched for information about biobanks in the past. Those who are better informed about biobanks and willing to participate in biobanking activities are concentrated in Northern Europe – in Sweden, Finland, and Iceland. Therefore it is obvious that the perception for biobanking and research is directly related to the level of knowledge and exposure to the subject. In the USA [2] 30 years and older groups were favorable toward participating in biobanking if their concerns were addressed, such as confidentiality and consent issues. In Canada on the other hand the public has a positive attitude towards biobanking [3] with 60 % of cancer patients agreeing to one time consent but almost all want their results back [4].

In the Middle East perception also varies from one country to another. For example in Jordan the public was surveyed and attitude towards medical research was positive in that 88 % of the participants thought research was advanced in Jordan and 64 % were willing to participate in biobanking [5]. Other Arab countries in the Middle East where similar studies were done showed that 89 % of those surveyed in Egypt had a positive attitude [6] while this figure is much lower in Saudi Arabia where only 69 % had similar attitude [7, 8].

The willingness to participate in biobanking also varies from one country to another in the Middle East and also varies with the type of specimen to be donated or with the risk involved in the procedure. In Egypt for example 82 % would donate blood for research but only 69 % would volunteer for studies involving tissue. This is in contrast with donating blood to be banked for future research where only 44 % of the study population in Egypt would agree to participate [6]. The reason for this is probably related to the unidentified nature of the future research.

On the other hand in Saudi Arabia 87 % are willing to donate blood for research and 70 %

would allow the use of excess tissue left over from surgical specimens [7, 8].

Regarding factors that might influence the public participation in biobanking such as the presence of an informed consents and other ethical issues, there are also regional variations for example majority of those surveyed in Egypt felt there was no need for informed consent to allow the use of left over specimens in research [9] while 65 % of surveyed Jordanians said that the presence of informed consent would not influence their decision to participate in biobanking. In addition the majority (75 %) of Jordanians would agree to an open consent and only 15 % want it to be disease specific [5]. In contrast in Egypt 40 % of those surveyed felt that consent should have an option to restrict future use to a specific disease and 54 % felt that there was no need to have an option for such a restriction [6].

The lack of monetary benefits or direct health benefits would have a negative influence on only 15 % and 13 % of Jordanians [10] and Egyptians [6] respectively regarding their decision to participate. The right to withdraw from research would only influence 31 % of Jordanians and similarly 29 % of Egyptians survey participants regarding their decisions.

One important factor that seems to negatively influence the decision to participate in biobanking is the lack of return of personal results for as much as 47 % of those surveyed in Jordan highlighted this opinion [10]. On the other hand 89 % of Egyptians [6] surveyed want to be notified of their results while only 12 % of the Saudis want that done [8].

In conclusion there is a positive attitude towards research in general and positive attitude towards biobanking with some variation from country to another in the Middle East. Variation seems to be related to lack of knowledge or exposure within the same country or between countries. Education of the public regarding biobanking is paramount to the success of any future biobank.

11.2.1 The Experience of Establishing the First Cancer Biobank

The establishment of our cancer biobank (KHCCBIO) took place on multiple stages. The first stage was to create a consortium of partners. KHCCBIO consortium consisted of partners from four distinct organizations with complementary and diverse skills which supported all the key pre-requisites to establish a first-of-its-kind, state-of-the-art cancer biobank in Jordan. The consortium constituted of the project coordinator which was represented by KHCC located in Amman, Jordan and three other EU partners which were represented by Trinity College Dublin (TCD), Ireland; Biostór, Ireland and Accelopment AG, Switzerland. Taken together, the consortium had the necessary skills, experience and expertise in pathology, tissue biobanking (both medical and commercial), regulatory processes, quality management systems and accreditation and project management [11].

11.2.2 Ethical, Legal and Social Issues (ELSI) Policy Development

Protecting the privacy and confidentiality of patients is extremely important for the success of any biobank. Hence, the second step in establishing the KHCCBIO bank after forming the consortium was to establish ethics related policies and procedures which governed data privacy, confidentiality and sample ownership among other ethical issues in biobanking.

Since KHCC has a well established and functional institutional review board (IRB) with approved IRB Policies & Procedures [12]: it was relatively an easy task to establish KHCCBIO bylaws manual which defined, in detail, policies regarding the ethical issues involved in the use of data or tissue repositories for research purposes and set conditions whereby data and specimens may be accepted and shared through the use of

material transfer agreements (MTAs). It also defined rules for access to the bio-resource, ownership of biological samples and ways to ensure the highest standards of ethics and governance. Within this manual, it is clearly stated that

- Written informed consent should be obtained from each donor-subject. Included among the basic elements of the informed consent there should be a clear description of, the operation of the biorepository, the specific types of research to be conducted, the conditions under which data and specimens will be released to recipient-investigators, and procedures for protecting the privacy of subjects and maintaining the confidentiality of data.
- Informed consent information describing the nature and purposes of the research should be as specific as possible when applicable.
- Where human genetic research is anticipated, informed consent information should include in-formation about the consequences of DNA typing.
- Informed consent documents may not include any exculpatory language through which subjects are made to waive, or appear to waive, any legal rights.
- Formal written agreements need to be established stipulating that, the repository will not release individual identifiers to investigators, the investigator will not attempt to identify or contact subjects through any means, the investigator(s) will use the data or tissue for research purposes only, as specified to and approved by the IRB. Any additional use of repository material will require prior review and approval by the IRB. The investigator(s) must report promptly to the repository any proposed changes in the research project and any unanticipated problems involving risks to subjects or others. The investigator(s) will comply with any conditions determined by the IRB to be necessary for the protection of subjects.

The KHCCBIO bylaws manual embarked on many of the issues that need to be addressed before a biobank can be established and has paved the way for identifying many of the ethical issues which might arise during the development of a cancer biobank [13]. For example, in order to obtain most of the relevant information from each consented patient, the means by which the data is obtained, classified and stored must be clearly defined in order to protect the privacy of the patient. Data must be obtained using appropriate consenting, and must be recorded in such a way that enables it to be compared with other data. Data collection, including follow-up information, should be coordinated between various research centers, and a minimum clinical dataset should be defined. Most importantly, the security of the data obtained in relation to coding patient information is critical. Furthermore, it is essential that the biobanked samples can be tracked at all times. While several documents produced by international experts outline best practices for data collection and management, standards for biobanking data collection are used at KHCCBIO, based on the guidelines set out in the National Cancer Institute Best Practices for Biospecimen Resources [14]. It is well known that all guidelines for best practices state that all relevant clinical data associated with samples must be collected in a way that is in keeping with the relevant regulations. A unique identifier is assigned to each sample or a combination of identifiers using a barcode system. The informatics system responsible for the data management must be strong, secure and reliable, and have the ability to support all biobank operations. Furthermore, in compliance with the Molecular Medicine Ireland Guidelines for Standardized Biobanking the biobank data management system must have the capacity to track all phases of sample collection, processing and distribution [15]. The database must also be located on a secure site and have the appropriate security in place for access by biobanking personnel only. In addition to its role in the storage and retrieval of data, it must be able to facilitate monitoring and reporting on sample quality [11].

11.2.3 Implementing Quality Measures by Developing a Quality Management System

Prior to the establishment of KHCCBIO, KHCC had a strong appreciation for Quality Management Systems. In 2006, KHCC was awarded the Joint Commission International (JCI) accreditation, the international arm of the Joint Commission on Accreditation of Health Care Organizations (JCAHO) USA, which was subsequently re-accredited in 2009, and 2012. In December 2007, KHCC was awarded Disease or Condition-Specific Care (DCSC) accreditation for its Oncology program, while in 2009, the Department of Pathology and Laboratory Medicine (DPLM) was accredited by The College of American Pathologists (CAP) and to this date the CAP accreditation was renewed three times for the department of Pathology and Laboratory Medicine. Furthermore, the entire KHCC facility was awarded local accreditation from the Health Care Accreditation Council of Jordan (HCAC).

As part of the on-going work of the KHCCBIO consortium, an ISO standard Quality Management System (QMS) was designed to prevent, detect, and correct deficiencies that affect the quality of procured, and processed biospecimens and would result in consistently more uniform samples. This resulting QMS required, among other things, procedures for appropriate handling, labeling and record keeping for the procurement, processing, preservation, extraction, storage, distribution and disposal of human specimens. As such, KHCBIO is required to retain records concurrently with the performance of each significant step in this process. A record management system was established and records will be maintained both electronically, and as original paper records or as true copies, 10 years after their creation and also for contracts, agreements, and other arrangements with other facilities. Procedures were also maintained for the prompt review, evaluation, and documentation of all adverse occurrences and complaints, and the investigation of these as appropriate.

The QMS will help KHCCBIO in maintaining an adequate organizational structure and provide the personnel with the necessary education, experience, training and re-training to ensure competent performance of procurement, processing, preservation, testing, storage, distribution and disposal of biospecimens. For example, the KHCCBIO Quality System requires that all equipment, systems and processes that critically affect the quality of the samples to undergo qualification and validation. Moreover, the QMS will require that all computer software used as part of tissue donation, procurement, processing, storage or distribution or for tracking, or for maintaining data relating to donors and other activities, to be appropriately validated. Furthermore, equipment used in the procurement, processing, preservation, storage and distribution of cancer biospecimens will be suitably located and installed to facilitate operations, including cleaning and maintenance. The QMS also requires procedures and schedules for the calibration of equipment, inspections and records. KHCCBIO will establish and maintain procedures for receiving supplies and reagents used in the procurement and processing of biospecimens. These items would be qualified to meet specifications designed to prevent circumstances that could affect the quality of the procured tissue samples and the ensuing results. The QMS will implement a system of "Change Control" whereby, when procedures are qualified, they cannot be changed without prior approval. Such changes would be verified or validated, and approved by the appropriate personnel before implementation [16].

11.2.4 Validating Sample Collection, Storage and Distribution

The third step in establishing the KHCCBIO bank was to implement quality measures. Through this stage KHCCBIO team in collaboration with its international consortium established an ISO-compliant quality management system and wrote standard operating procedures (SOPs) for collecting, processing, storing and distributing human tissue and blood. These SOPs were

reviewed and revised by KHCCBIO quality officer and approved by KHCCBIO director.

Specific SOPs for a variety of different biospecimens were developed such as SOPs for fresh and frozen normal and tumor tissue, formalin-fixed paraffin- embedded tissue (FFPE), blood plasma, serum and white cells/buffy coats. Special constraints must be taken with each sample type, hence, it was recommended to follow international guidelines such as those outlined in the National Cancer Institute Best Practices for Biospecimen Resources [14] and ISBER's 2008 Best Practices for Repositories document [17]. These include the timing incurred at each stage of specimen collection and processing, up to the point of storage. The ISBER guidelines recommend that pilot studies or feasibility studies be carried out to identify any problems associated with the collection and processing of particular sample types [17].

Quality control assessments for biospecimen quality should be adequately recorded in relation to the methods employed and the results obtained. This quality assessment process for sample collection, processing and storage must also be standardized and should include the time recorded from the time of the cancer resection for examples, to the freezing of samples in the cancer biobank [18].

Moreover, quality control for collected tissue samples must include traditional Haematoxylin and Eosin (H&E) staining of sections for each specimen collected in addition to the steps mentioned above. DNA and RNA integrity isolated from blood or tissue samples collected as part of the biobank, should all be tested as is the practice in other established cancer biobanks, as it is the case in the Spanish National Tumor Bank Network and the Wales Cancer Bank [19, 20]. During the collection process, it is crucial to maintain diagnostic integrity; therefore, a pathologist should supervise the tissue procurement process. The pathologist must also review all patient tissue specimens to determine what material can be made available for research and the optimal samples and number of aliquots to be taken. Blood and other body fluids, not required for diagnosis can be collected from the same donor in accordance with approved protocols and do not require pathologic review. Importantly, where samples are to be aliquoted, there are a number of standards to be considered in relation to freezing and thawing (e.g. the rate of cooling, storage, handling and reconstitution). In addition, the retrieval of specimens from storage must adhere to strict protocols for sample inventory and tracking. There should be an appropriate inventory system and SOPs for sample retrieval along with checklists and other forms which are specifically designed to document the process.

The processes of validation of sample collection, processing, storage and dissemination was carried out in collaboration with our EU-partners in Ireland Trinity College Dublin (TCD). In order to collect specimens for the validation procedure patients were consented prior to sample collection. A biobanking label was then assigned to the front of the patient's medical chart for further identification of the patient as a biobank patient at time of surgery. On the day of surgery, personnel on biobank duty were informed by the operating theater of the sample upon resection. The time from collection and transport of the tissue from the operating theatre to final storage was recorded. It is critical that this time is kept to a minimum so as to avoid the possibility of sample degradation or otherwise. Based on what proportion of normal and tumor tissue is available, the pathologist selected an appropriate amount of tumor and normal tissue. These tissues were then further divided and immediately (i) flash frozen in liquid nitrogen, (ii) preserved in RNAlater™ or AllProtect® and (iii) inserted into cryomoulds in OCT and subsequently flash frozen in liquid nitrogen. Tissue samples preserved in the stabilization reagents RNAlater™ and AllProtect®, were stored at 4 °C overnight, after which time the tissues were then removed and stored at −80 °C. Tissues were stored until a pathological diagnosis has been confirmed. After this time, tissues can then be processed into blocks by a histo-pathologist and stored until further use (Fig. 11.1) [11].

The distribution of tissue from the biobank will be tightly regulated and evaluated by the internal ethical review of KHCCBIO based on a

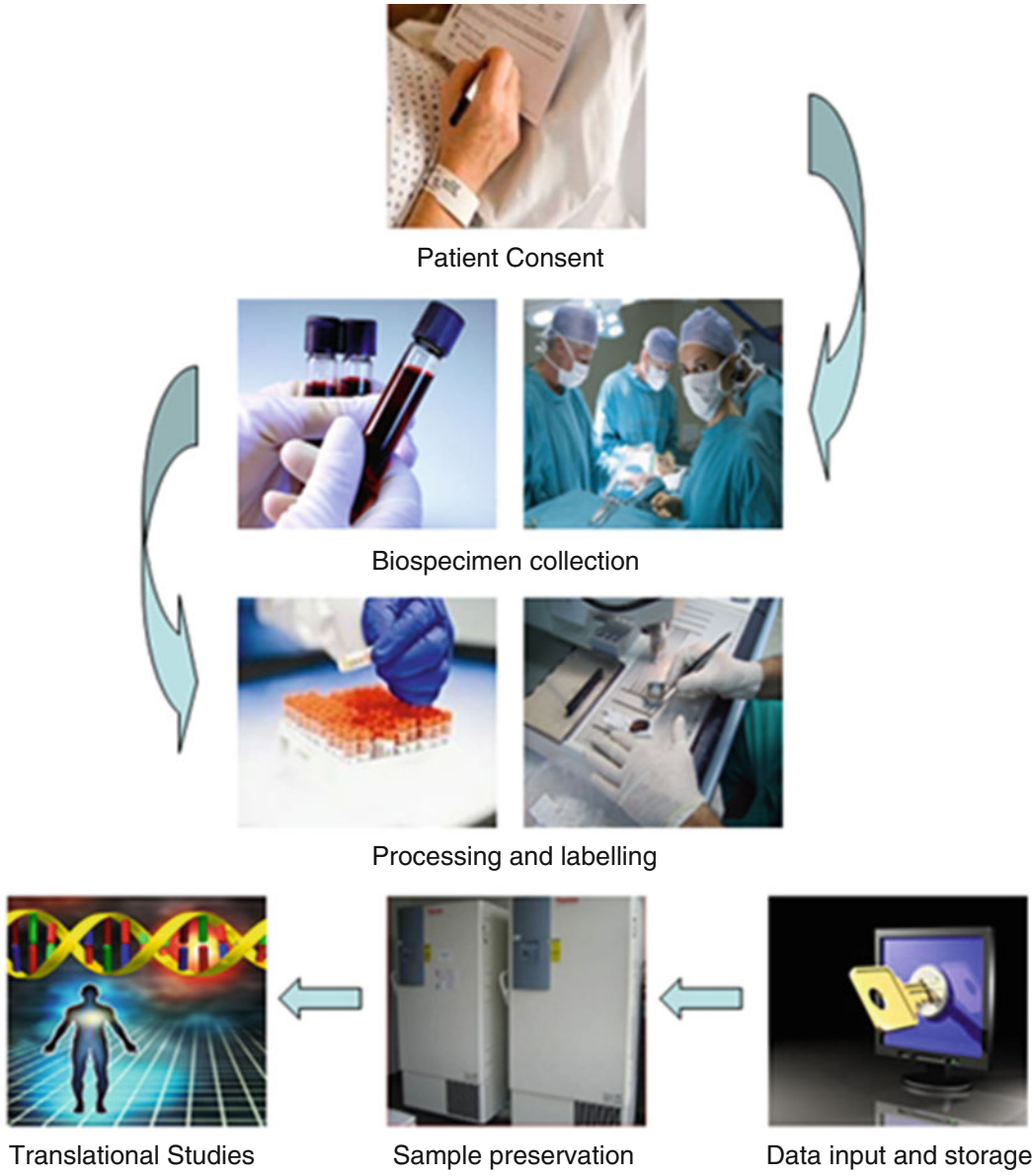

Fig. 11.1 Workflow of the various stages involved in the procurement, processing and preservation of biobank samples. Following initial patient consent, blood and tissue samples are procured and processed following surgical resection of the tumor. Specimens are appropriately labeled and stored under tightly regulated storage conditions. Patient clinical-pathological data is retained in a password protected, secure database within the biobanking facility. These biobank samples may ultimately be used in translational studies, such as the identification of novel biomarkers or targets in the development of personalized medicine for cancer patients (Reproduced with permission from Barr et al. [11])

number of criteria such as the relevance of the study for which the biospecimens are required, the amount of sample requested, together with the scientific validity of the study in question. Following such criteria allows the biobank to accurately track the amount of sample available in the biobank. One must consider carefully however, the associated clinicopathological data,

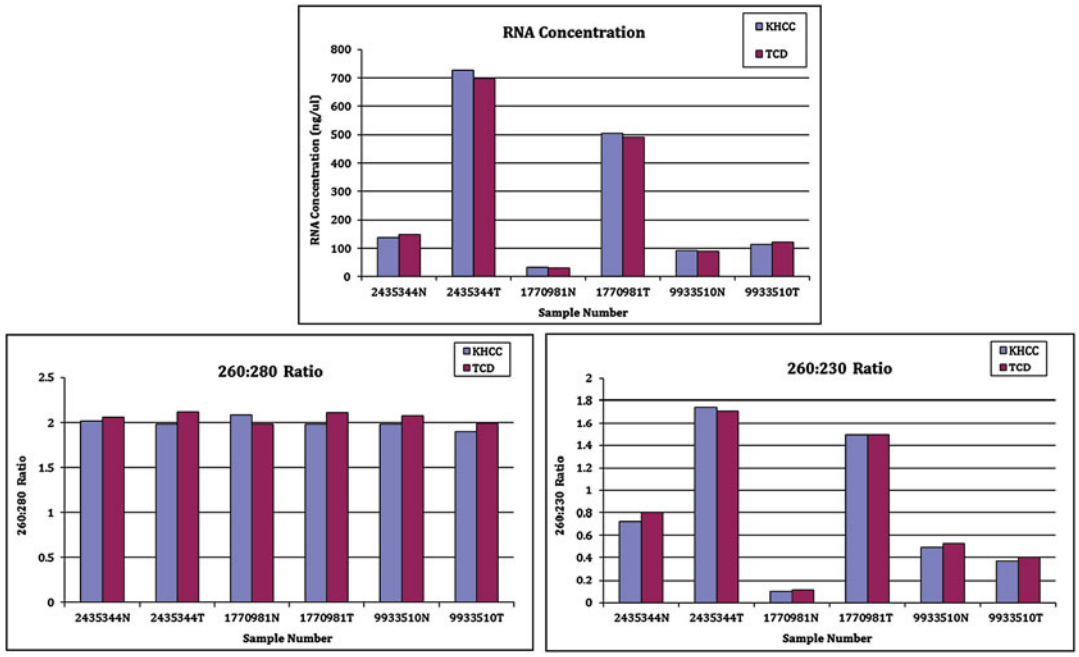

Fig. 11.2 Validation of transported tissue RNA. Integrity of shipped RNA samples extracted from normal and tumor tissues was validated using a liquid nitrogen dry shipper between Europe (Ireland) and the Middle East (Jordan). RNA concentration and integrity was measured using 260:280 and 260:230 ratios at TCD prior to shipment, and upon receipt of sample at KHCC (Reproduced with permission from Barr et al. [11])

where these are bound by the provisions approved by the IRB of both the institution where the biobank is housed and the requesting investigators ethical review board.

To this point all the previous steps were carried out on TCD premises. Therefore, in order for KHCCBIO to validate the transportation of RNA isolated from patient tissues between the lung cancer biobank at St James's Hospital & Trinity College Dublin and KHCCBIO we used a liquid nitrogen dry shipper. Prior to the shipment of samples from Dublin, RNA concentrations and integrity were validated by NanoDrop® Spectrophotometry. Upon arrival of the dry shipper containing the RNA samples in Amman, Jordan, RNA concentration and integrity were measurements were repeated using a similar NanoDrop® Spectrophotometer as part of a blinded sample validation cross-study between the two institutions [11]. A comparison of RNA yield together with 260:280 and 260:230 ratios was made on the same normal and tumor tissue samples, and demonstrated a high level of consistency in RNA readings between both biobanks (Fig. 11.2). This preliminary validation study further highlighted the use of the liquid nitrogen dry shipper as a successful way for transporting biospecimens, in particular nucleic acid components from patient tissues, between Europe and the Middle East. Furthermore, the shipper is validated to maintain temperatures for 2 weeks which overcomes any potential issues with delays in customs clearance [11].

11.2.5 Biobank Safety and Security Measures

Best practices in the safety, security and back-up of biobank samples must be followed when assessing the Biobank's safety and security requirements. Such practices are outlined in ISBER 2008 Best Practices for Repositories for

example [17]. Since the purpose of the biobank is the safekeeping of the biospecimens collected, many aspects of facility design which may affect the quality of the samples must be considered, such as protection against fire, storage temperature and air flow. In addition, monitored security systems must be installed in addition to continuous monitoring of alarms. These systems must be designed to enable a number of responsible individuals to respond to an alarm in a timeframe that either prevents or minimizes the loss or damage of the biospecimens [11, 21]. The biobank material should be housed in a restricted area which is operated using key-ID-coded access with the date, time and ID information captured when the door is opened.

One of the risk management procedures for electrical power cuts is to have a back-up power supply in place for all freezers containing biobank samples. Best practice recommends that computer systems and electronic systems such as freezer controllers should be protected by an uninterruptible power supply (UPS) system [21]. Freezers and fridges should also be monitored daily. In the case of liquid nitrogen storage, a continuous supply should be readily available, while the room containing the liquid nitrogen should be monitored and alarmed [11].

KHCCBIO implemented all of the aforementioned safety measures. Security cameras were installed to continuously monitor the entrance, corridors and the entire biobank premises, biobank lab doors are operated using key-ID-coded access, and backup generators are installed along with UPS system in case of electrical shortage. All these are connected to alarms which are also monitored by KHCC security personnel and BMS engineers. All relevant personnel safety measures have also been implemented. Protective clothing, gloves and face protection, in addition to oxygen sensors (both room and personal monitors) have been installed. Procedures for maintenance, repair, and calibration of equipment are also in place. For personnel safety, national guidelines for health and safety in the workplace were always followed [11, 22].

11.2.6 Qualification and Validation of Equipment and System's Infrastructure

Validation and training plans, policies and procedures were also developed by KHCCBIO staff; in collaboration with our international partners. During the course of establishing KHCCBIO, the installation of essential biobanking equipment and systems was required, in addition to performing a documented Installation Qualification (IQ) and Operational Qualification (OQ) to verify that the biobanking equipment and systems meet user and ISO requirements. Based on the Validation Plan defined within the project, one of the international partners, Biostór, provided KHCC with draft User Requirement Specifications (URS), IQ and OQ protocols and report formats for the identified equipment and systems based on the existing Biostór-quality management system. KHCCBIO staff was subsequently responsible for revising, adapting and approving the drafts and performing the equipment testing required. A Master Project Plan of document deliverables was developed for the infrastructure qualification in addition to a validation report following qualification of the biobanking cryoshipping equipment and −80 °C storage systems [11].

11.2.7 Personnel

In order to achieve our goals a dedicated team was hired on either a fulltime or part time bases. The team included a director who is a pathologist, a deputy director and chief quality officer, a junior pathologist, two technologists and a document controller. Staff specific policies, job descriptions and competency assessment schemes were developed and implemented.

11.2.8 Dissemination and Impact on Health and Society

The proposed KHCCBIO project has been conceived to create a world-class, ISO accredited biobank at King Hussein Cancer Centre (KHCC)

in Amman, Jordan to be used for research into new treatments for cancer. While the ultimate aim of the biobank is to improve overall patient care, it is essential that patients, as well as the general public, understand the purpose of the biobank and the role that patients and the public alike can play in making it a success in paving the way forward in the development of improved diagnosis and novel cancer therapies. Two-way communication is therefore essential as part of this initiative. Most importantly, patients and families are fully informed in the hospital setting in relation to issues such as consent and privacy [12]. It is necessary that people understand that each of us must play his/her own part if we are to maximize the future benefits for the people of Jordan and its neighboring countries which would be created as a result of the establishment of the biobank. Therefore, in order to enhance public awareness, a project website has been developed by KHCC (khccbio.khcc.jo) consisting of a public and private area. Parts of the website that are accessible to the general public contain relevant information on KHCCBIO, ongoing developments of the biobank, in addition to general educational information on the biobanking of cancer tissues and other biospecimens targeted to tissue donors and their families, media, stakeholders, and project ambassadors. Secured areas (extranet) on the other hand are restricted so that current and future project partners can share and disseminate related knowledge and data. The publicly accessible KHCCBIO website will hopefully lead to higher awareness on the importance of tissue biobanking in the society and will provide an update to researchers in both the scientific and medical communities, on the progress of KHCCBIO and its implications in the development of improved treatments for cancer patients [11, 12]. Furthermore, local and national media including radio, television and press as well as online media campaigns were used to deliver the core message of KHCCBIO in a consistent and transparent fashion.

We envision that in the long-term, KHCCBIO will have an immediate impact on the health of Jordan and the Middle East through the availability of biospecimens from cancer patients. In the short term, the initiative will add value to, and increase the scope of clinical trials which may directly benefit all patients. As a result of clinical trials and research activities, prognosis and care will be more consistent, standardized and precise. Having such an accredited biorepository, will help reduce the time required for drug development, ensuring cancer patients have more realistic access to novel cancer treatments sooner rather than later. The availability of better drugs will be complemented by improved diagnostics through the availability of novel biomarkers as a result of research on biobanked samples. Thorough validation and optimization of new technologies using biobanked samples will ensure the appropriate diagnosis and classification of tumors, which will provide the basis for administering more effective personalized treatments as demonstrated by the development of novel drugs such as GLEEVEC® (Imatinib mesylate) and Herceptin (Trastuzumab) [23]. This shift towards more personalized medicine will allow the use of therapies that are best suited to the individual patient, thereby improving efficacy and reducing adverse effects.

While the ultimate strategy in developing biobank resources worldwide is to improve on the prevention, diagnosis and treatment of cancer of disease and to promote the health of society [24], the establishment of KHCCBIO would represent an important economic investment for Jordan. The knowledge economy requires that information be available to industry, and a biobank is an extremely rich source of such relevant information. The knowledge economy also requires a lively academic research environment, feeding into the translation of research results into meaningful products. Research arising from studies using biobank material will lead to knowledge generation and the development of research skills, and will contribute to the building of research capacity. Such a valuable infrastructure could potentially see an increase in industry research and development which would further strengthen the economy

and attract additional pharmaceutical companies and increased foreign investment to Jordan. In order to ensure that research efforts are not duplicated, collaboration not only within Jordan but also internationally should be promoted. A number of regulatory and ethical difficulties with regard to exchange of tissue and data remain however. This has been highlighted by the TuBaFrost group [25] and others. Efforts such as the European Bio-Banking and Biomolecular Resources Research Infrastructure (BBMRI), which was initially funded as a European infrastructure under FP7, are aiming to harmonize and co-ordinate existing infrastructures, develop new tools and technologies, and facilitate studies of unprecedented statistical power by bringing together fragmented collections in over 20 different countries [26]. The BBMRI-ERIC Inauguration Conference was held September 16, 2013 in celebration and recognition of the inauguration of the Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) implemented under the European Research Infrastructure Consortium (ERIC) legal entity.

11.2.9 Health Impact

For the first time in the Middle East, KHCC has pioneered the concept of Multidisciplinary Clinics (MDCs). MDCs are highly specialized clinics in specific types of cancer. Each Multi-Disciplinary Clinic is comprised of a team of health care specialists that include a minimum of a medical oncologist, a surgical oncologist, a radiation oncologist, and an oncological pathologist and a radiologist who together set the patient's treatment plan, and supervise their care. To date, the Center has established the following Multi- Disciplinary Clinics: Bone Marrow Transplantation Clinic, Breast Cancer Clinic, Lung Cancer Clinic, Thyroid Cancer Clinic, Sarcoma Clinic, Head and Neck Cancer Clinic, Gastrointestinal Malignancies, Gynecological Malignancies, Genitourinary Malignancies, Ocular- Oncology, Lymphoma Clinic, Myeloma Clinic, Leukemia Clinic, Neuro Oncology Clinic. These MDCs will feed KHCCBIO with samples representing every therapeutic area of pharmacologic interest, thus opening the horizon for a vast variety of collaborations with the industrial sector [12, 27].

A Global Task force was formed in 2009 by Harvard University where the director of KHCF was named as honorary co-president and the CEO/Director General of KHCC was named to the Global Task Force's Technical Committee. The Global Task Force was established with the mandate of designing and implementing a scheme for expanding access to cancer education, prevention, detection and care in the developing world.

Furthermore, KHCC developed and maintains a cancer registry where two cancer registrars that were certified by the National Cancer Registrars Association (NCRA) in the United States in December 2009, are responsible for this task. These registrars are the only two certified cancer registrars currently working in Jordan. KHCC's Clinical Research and Registration Office (CCRO), the only office of its kind in the country, captures a complete history of all KHCC patients. The Registry's collective data is researched and used in the management of cancer, and ultimately, cures [27]. In addition to the valuable associated data, KHCCBIO will provide histology slides used for clinical diagnoses in addition to the cryo-preserved tumor specimens, as well as secured related test validation protocols used for procurement and processing of the samples. One of the main goals for KHCCBIO is to reduce risk and the cost of therapeutic drug development for our partners by translating clinically relevant molecular response marker data into high-value knowledge while fully integrated into existing pharmaceutical development processes and programs.

11.2.10 Economic Impact

KHCCBIO also aims to drive economic growth, create jobs for Jordanians and enhance the business climate in the country. KHCCBIO aims to expand into a national wide biorepository bank which will increase the sector's competitiveness by increasing the number of grants and training fellowships applied to the European Union. By both supporting improvements in the business environment and providing assistance to expand innovation and productivity in Jordanian businesses, it supports the main objective of building up the private sector as a powerful engine of economic growth which increases stability in the region and provided sustainability to the project [12].

Jordan Private Hospitals Association (PHA) figures indicate that 1,200 of the 20,000 doctors registered at the Jordan Medical Association live and work abroad, 250 in the US [28]. *KHCCBIO* bank will attract more physicians to come back to Jordan and collaborate with their institutions worldwide due to the availability of quality preserved material worth performing research on for the benefit of patients and the development of personalized medicine. In addition hundreds of Jordanian scientists are currently working abroad in Europe and the USA. Many of these are well established in the fields of biomedical research and will be willing to come back and continue their research in collaboration with their mentors abroad.

Anticipated collaboration of KHCCBIO with biopharma partners can also enable these companies to select what disease targets to avoid for new drug candidates and pick the best new cancer indications for experimental drugs to maximize clinical trial budgets. We envision that the future plans for KHCCBIO after it becomes nationwide approved biobank to start collecting discrete cancer samples that are preserved as living single-cell suspensions, stored at −180 °C, to ensure availability for cell line propagation profiled against a panel of drugs to be available to researchers and pharmaceutical company customers in order to select tumor cells of interest based on drug sensitivity/resistance or to be used for ex-vivo treatments.

11.2.11 Utilization of KHCCBIO Bank by Middle East Countries

We further anticipate that KHCCBIO will make special tumor cell lines, prepared from our ethnic background that covers the entire Middle East region commercially available which will give pharmaceutical companies and researchers in Europe and the world opportunities to develop clinically sound hypotheses that will help identify new diagnostic and therapeutic targets. In order to fully exploit this potential and to have the widest possible outreach, KHCC collaborates with a number of potential users of the biobank from the Middle East will be identified and given access to the biobank facilities and resources.

We believe that KHCCBIO will increase Jordan's medical achievements, thus increasing its potentials and competitiveness in the scientific world. KHCC's experience as one of the leading medical centers in the region and one that provides integrated qualitative services that attract patients from the region and the world makes it easy for researchers from Europe and the US to establish joint research with local researchers to make use of the unique stored samples which KHCC will help preserve based on ISO high quality standards.

For the reasons mentioned above, Jordan is also considered to be a favorable hub for the scientific and economic international conferences such as the American Association for Cancer Research (AACR), WHO and UNESCO meetings, Middle East and North Africa region (MENA) conferences, and Davos Forum's Annual Meeting. All these conferences can be a useful resource to disseminate information to the public on KHCCBIO and its services and potential collaborations. It will also allow individuals and communities to benefit from the educational

opportunities offered in order to achieve a concrete improvement in social and economic aspects. It will use the educational process as a mean to achieve sustainable development through policies and educational development plans in order to eventually overcome whatever obstacle halting the development of Arab communities and the region in the medical scientific research and provides a focal point for collaboration with the European Countries.

11.2.12 Collaborations at a Global Level

The King Hussein Cancer Center has also developed cooperative relationships with several world-renowned cancer centers. This enables the Center to benefit from the latest in treatment protocols and exchange expertise and staff with these prominent institutions and allows our collaborators to benefit from our samples collected in our KHCCBIO bank. These partnerships include: M.D. Anderson Cancer Center (MDACC), Texas USA, National Cancer Institute -Cairo, Egypt, St-Jude's Children's Hospital, Tennessee USA, MOFFIT Cancer Center, Florida USA, Lombardi Comprehensive Cancer Center (LCCC), Georgetown University USA, Hospital for Sick Children, Toronto Canada, Augusta Victoria Hospital, Jerusalem Palestine, American University of Beirut, Beirut Lebanon, Royal College of Surgeons in Ireland, Medical University of Bahrain. The wealth of knowledge and expertise now housed within KHCC enable the Center to provide highly specialized unique programs in cancer care such as Bone Marrow and Stem Cell Transplantation. Furthermore, several pharmaceutical companies have established clinical trials at KHCC such as Novartis and others. Moreover, KHCC became a member in the Worldwide Innovative Networking (WIN) in personalized cancer medicine initiated by the Institut Gustave Roussy (France) and The University of Texas MD Anderson Cancer Center (USA) which is a non-profit non-governmental organization bringing together 22 cancer centers and industry partners from five continents to address the challenge of increasing the efficacy of cancer diagnostics and therapeutics.

The establishment of KHCCBIO represents an ideal opportunity to create a bioresource that can be used to further our understanding of the molecular and genetic factors that predispose this diverse ethnic Middle Eastern and North African population to cancer.

11.3 Conclusion

We described in this article, the procedures, challenges and outcomes of establishing the first cancer biobank in Jordan, KHCCBIO. KHCCBIO was established to facilitate a biorepository of blood and tissue from cancer patients, through the collection, processing, testing and preservation of appropriately-annotated samples for subsequent use in translational research efforts, both in the Middle East and Europe. In collaboration with its partners, TCD, Biostór and Accelopment AG, a solid infrastructure has been put in place for KHCCBIO. This collaborative effort not only involved KHCCBIO and its partners, but also the relevant ethics and advisory committees, IT personnel and financial administrators. KHCCBIO covers a range of support activities that are in place to continue to operate and maintain sustainably a cancer biobank. The biobank related activities will incorporate all current international guidelines and best-in-class practices under an approved Quality Management System to procure, process and store biospecimens for the benefit of researchers in Jordan, its neighboring countries and researchers throughout the rest of the world. The KHCCBIO banking project has been conceived to create a world-class, ISO accredited biobank in the King Hussein Cancer Centre, Amman, Jordan and will potentially have a major impact by contributing to medical advances by providing high-quality human biospecimens and associated clinicopathological data to cancer research communities world-wide.

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Nursing and Biobanking

12

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Abstract

Nurses are a pivotal component of the translational research movement and apply scientific discoveries to the healthcare and clinical practice fields. Biobanking is also an important factor in furthering translational research by providing biospecimens and related clinical data to the research community. The effectiveness of any biobanking effort necessitates the enrollment of large numbers of diverse participants, which signifies a need for the nursing profession to secure the knowledge necessary to impact biobanking practices and to promote participant advocacy. In addition, biobanks provide the volume, variety, veracity, and velocity of data that can address the challenges of nursing research. Nurse scientists, research nurse coordinators and clinical research and practice nurses must be informed about the various benefits and risks associated with biobanking in addition to ethical issues surrounding informed consent, participant privacy, and the release of research results. Ultimately, nurses need to possess competencies to facilitate biobanking practices both at the research bench and at the point of care.

Keywords

Nursing practice • Biobanking • Informed consent • Nursing competencies • Ethics • Genetics and genomics

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12.1 Introduction

Nurses are at the forefront of the translational research movement, promoting the transfer of basic science discoveries to healthcare applications and clinical practice [1]. Biobanking has also played an important role in furthering translational research by providing large numbers of readily accessible biospecimens and related clinical data to the research community. Furthermore, biobanking has revolutionized the methods in which data are collected and obtained as well as added a new dimension to nurse-research participant interactions. Ultimately, nurse scientists, research nurse coordinators and clinical research and practice nurses must be knowledgeable about the various benefits, risks, and ethical issues associated with biobanking.

Researchers, including nurse scientists and nurses involved in various aspects of research, need to be aware of the potential benefits and risks associated with biobanking and need to be involved in protecting the rights of the participants. The benefits of biobanking include access to biospecimens and related clinical data for use in research studies, at times circumventing the necessity for an individual research project to recruit their own research participants and use larger amounts of research funding. Biobanks may also provide genomic data (e.g., whole exome sequencing, single nucleotide polymorphism (SNP) analysis), which may be very useful to researchers conducting genetic studies and genome-wide association studies. Furthermore, biobanks promote and facilitate broad avenues of research, potentially benefiting the health of society. In addition, the inclusion of nursing-relevant data may be geared towards answering nursing research questions [2].

The ethical issues associated with biobanks include but are not limited to protecting the privacy of the participant and the potential for discrimination against the biobank participant. Successful biobanks call for adequate human subjects' protection and data privacy protection necessitating the education of professionals, including the nursing profession [2]. Overall, biobanking produces many benefits associated with its incorporation into translational research; however, risks, primarily to the participants, are also involved in biobanking and should be considered by researchers, including nurse scientists, in developing and utilizing biobanking practices.

Three essential ethical considerations of biobanking or research involving human subjects are protecting the identity of the participant and data privacy, ensuring that the research participant has provided informed consent, and the potential disclosure of results or incidental findings [3]. Components to the informed consent process for potential biobank participation necessitates language in the informed consent which addresses identity protection and data privacy measures, allows for the sharing of the contributed data in future unknown studies and discloses the potential for discovery of future results or incidental findings, all of which may be challenging to explain to potential participants. Nurse scientists, research nurse coordinators, and clinical research and practice nurses all play important roles in shaping and exercising biobank practices, especially principles involving advocating for and protecting the rights of the research participants, including the informed consent process. Nurses should address these ethical considerations of biobanking by educating themselves, interdisciplinary team members, participants, family members, and the public about biobanking; by serving as participant, family, and public healthcare advocates; and by engaging in healthcare policy that influences biobanking practices [2].

As an integral component of the translational research movement, the genomics revolution has greatly impacted current research and influenced biobanking practices. Because a biobank may serve as a virtual or concrete repository for the storage of large numbers of human biospecimens and related clinical data, researchers conducting genetic research, which often requires a large collection of biospecimens, may use a biobank to gain access to large amounts of data. As the fields of genetics and genomics have grown, the nursing discipline recognizes the importance of incorporating the practices and competencies necessary to conduct cutting edge genetic research and incorporate genetic knowledge into

clinical practices in the genomic era, including biobanking practices. Specifically, these competencies include professional responsibilities such as knowledge, skills, and values and professional practice considerations such as nursing assessments, identification, referral activities, and provision of education, care, and support [4]. Biobanking and genetics and genomics are often intertwined practices; therefore, these competencies also apply to nursing professionals involved in biobanking. While not meant to be a comprehensive review, in this chapter, we will discuss various nursing roles influencing biobanking practices and research; outline competency standards for nurses, especially in the area of genetics and genomics and biobanking; and highlight pertinent ethical considerations for nursing research and practice involved in biobanking.

12.2 Nursing Roles Impacting Biobanking

With the advent of translational research, patients are increasingly solicited to as potential biobank participators to contribute personal data to supply biobanks [5]. The effectiveness of any biobanking effort necessitates the enrollment of large numbers of diverse participants representing populations of interest [2, 6]. This request of potential participants signifies a need for the nursing profession to respond by securing the knowledge necessary to impact biobanking practices and to promote participant advocacy. The characteristic ethical perspectives the nursing discipline brings to healthcare and research practices are valuable because biobanking practices should be implemented with the protection and benefit of the biobanking participants in mind. Influence from the nursing discipline is seen as an integral component of biobanking practices highlighting the discipline's position in promoting participant trust, conducting nursing research, and improving clinical practice [7]. Furthermore, including a nursing component in biobanking encourages biobank data contributions that are meaningful to nursing-relevant research questions as well as questions that specifically impact the nursing discipline and clinical practice [2].

Genetic and genomic science discoveries, repeatedly generated through the use of biobanked data, are redefining our understanding of disease and human health. Although the full potential of genomic healthcare and biobanking usability has yet to be realized, it is critical for nurses to understand and develop competencies in genetics and genomics to build a foundation for the application of nursing practices in biobanking. The application of genomic and genetic discoveries in healthcare continues to improve our understanding of disease prevention, diagnosis, and treatment, and biobanks function as a catalyst for these discoveries by supplying large numbers of human biospecimens and related data to researchers conducting genomic and genetic research, including nurse scientists [8]. Biobanks provide the volume, variety, veracity, and velocity of data that can address the challenges of nursing research such as the need for large sample sizes. Therefore, researchers and healthcare providers alike, including nurses, need to understand the benefits, risks, and ethical issues surrounding biobanking to effectively impact biobanking practices. Nurse scientists, research nurse coordinators, and clinical research and practice nurses all possess unique attributes that can contribute to and influence biobanking practices.

12.3 Nurse Scientists

Similar to scientists in other disciplines, nurse scientists focus on advancing scientific discoveries; therefore, their professional roles may also include generating new scientific knowledge using biospecimens, genomic data and clinical data obtained from biobanks. The National Institutes of Health (NIH)-supported Clinical and Translational Science Award (CTSA) program was launched in 2006, and many centers include a core biobank component as an important piece of the translational research process [9]. Nurse scientists continue to be actively involved in the

development and advancement of CTSA programs, including biobank components, requiring nurse scientists to emphasize collaboration and participation within biobanks and across disciplines within CTSA centers [1].

Nurse scientists may also use biospecimens and data retrieved from biobanks in their own studies; therefore, they need to be involved in the development of biobanking resources such as the collection of nursing-relevant data including bio-behavioral, health-related quality of life, cost of care and patient outcome data [1, 2]. In addition, nurses who are prepared at advanced educational levels should consider being actively involved in legislation and policy formation regarding biobanking practices. This governance includes biobanking use for genomic and genetic research, as well as generating research using biobanking data to advance the understanding of disease processes and human responses, symptom advancement, and self-management of these conditions [3].

12.4 Research Nurse Coordinators

Research nurse coordinators may engage in biobanking activities such as providing research staff oversight in participant recruitment; supervising data collection measures (including working with laboratory personnel to implement standardized biospecimen collection, handling, processing and storage protocols); direct data recording and management (including working with informatics personnel to standardized data processing and security protocols); ensuring compliance with institutional regulatory requirements and reporting; and preserving the integrity of biobanking protocols. Therefore, research nurse coordinators must understand, implement and follow ethical guidelines specified by the governing Institutional Review Board (IRB) and Committee for the Protection of Human Subjects (CPHS). For example, an important aspect of following established human subject protection guidelines may include research nurse coordinator involvement in the development of the informed consent for a biobank to ensure that biobank participants' rights are protected.

12.5 Clinical Research and Practice Nurses

With the increase in the use of biobanks in trials, it is critical for clinical research nurses to stay informed of the emerging ethical, clinical, and regulatory issues involving human subjects, including biobank participants. Clinical research nurses, frequently under the direction of nurse scientists or research nurse coordinators, often focus on fundamental biobanking activities to promote research endeavors, including obtaining participant informed consent and biospecimen and clinical data collection [10]. Therefore, it is imperative that clinical research nurses involved in biobanking have a comprehensive understanding of biobanking practices, including the ethical considerations surrounding the informed consent process and data integrity measures.

Potential participants are approached to partake in a biobank by a recruiter who may be a clinical research nurse. The initial informed consent process involves a purposeful and continuing exchange of information between the members of the research team and the potential participant throughout the research experience, and the clinical research nurse may be the representative of the research team who interacts with the participant to obtain the informed consent. While the clinical research nurse typically obtains the actual informed consent, the clinical practice nurse may perform multiple functions at the point of contact with the research participant, including reinforcing participant understanding of the informed consent process. Therefore, the clinical practice nurse may function as an extension of the biobanking research team [7].

Along with clinical research nurses, clinical practice nurses are often involved with data collection at the point of care; therefore, they often directly influence the quality of biospecimen and clinical data obtained for biobanking, and competent nurses ensure the validity of the collected biospecimens and phenotypic data. Once the patient has acquiesced to becoming a participant of the biobank, clinical research and practice nurses must understand and execute standardized techniques essential for the proper collection, handling, processing, and storage of biospecimens

and related clinical data. While nurse scientists and research nurse coordinators often establish and supervise standardized data protocols for data received by the biobank, clinical research nurses, and when appropriate clinical practice nurses, should be knowledgeable in these processes to facilitate the entry of intact data into the biobanking system. In acquiring data to deposit into biobanks, clinical research and practice nurses have an obligation to obtain quality data and related documentation.

12.6 Genetic and Genomic Nurse Competency Standards Impacting Biobanking Practices

Since the completion of the Human Genome Project in 2003, the importance of understanding and applying genetic and genomic information in nursing practice and nursing research has been recognized [11, 12]. As more patients are recruited into genetics and genomics studies that may involve biobanks, nurses will have increased opportunities to interact and be involved in the various aspects of biobanking operations and practices. To promote the understanding of genetics and genomics in the nursing field, the American Nurses Association (ANA) with the National Human Genome Research Institute (NHGRI), the National Cancer Institute, and the Office of Rare Diseases of the National Institute of Health organized a panel of nursing experts to establish a consensus on essential genetics and genomic competencies for all registered nurses [11]. After reviewing the available competencies, guidelines, and recommendations, "The Essential Nursing Competencies and Curricula Guidelines, 1st Edition" was published and endorsed by major professional nursing organizations including the National Coalition for Health Professional Educators in Genetics (NCHPEG) [11]. This guideline identified and implemented essential competencies to guide nurses in making the connection between bench genetic and genomic knowledge and point of care [4, 11].

The two domains of essential competencies identified for nurses are professional responsibilities and practice [4]. Professional responsibilities include the professional role of nursing practice as specified in the nursing scope and standards of practice [4]. Nurses at all levels, including nurse scientists, research nurse coordinators and clinical research and practice nurses, are required to be knowledgeable and competent in utilizing genetic and genomic knowledge and skills, and the first step is to recognize one's own attitude and values related to genetic and genomic discoveries [4]. In addition, essentials in the professional practice domain address nursing competencies such as nursing assessment, patient identification, referral activities, and patient support [13]. In the assessment areas, understanding the importance of genetics and genomics related to health prevention, screening, diagnosis, prognostics, and treatment are considered important competencies [11]. With the essential knowledge in professional responsibilities and practice, nurses are in a pivotal position to promote trustworthy biobanking practices in the genetic and genomic era today and in the future. The Educational Resources on Genetic Biobank Applications list provides educational resources related to genetics and genomics and biobanking, including topics of specific interest to the nursing profession.

12.7 Ethical Genetic and Genomic Considerations for Nursing Research and Practice Involving Biobanking

During participant recruitment for biobanks, nurses, frequently clinical research nurses, provide relevant information to the potential participant, obtain informed consent, and answer questions regarding biobanking practices. For genetic biobanks, the large-scale collection of biological samples and related clinical data raises serious concerns regarding the privacy and confidentiality of biobank research participants. To address these concerns, the Ethical, Legal, and Social Implications (ELSI) program was founded by the NHGRI in 1990 as an essential part of the Human Genome project [14]. Notably, the ELSI program focuses on ethical issues surrounding the design and conduct of genetic research,

including informed consent [15]. The paramount issues that are highlighted by the ELSI program associated with the informed consent process include the scope of the informed consent, informed consent content, protection of participant privacy and confidentiality, return of research results or incidental findings, termination of participation, custodianship and intellectual property rights, and access to and sharing of genotypic and phenotypic data [16]. Even though the purpose of ELSI was to proactively address the issues from genetic and genomic studies, biobanks practices are often included in the discussion, specifically related to broad prospective consent and return of significant or incidental results [17]. These ethical dilemmas addressed by the ELSI program pose novel challenges for nurse scientists, research nurses, and clinical practice nurses conducting genomic and genetic biobanking research [18].

Although the biospecimens stored in a majority of biobanks are de-identified to protect participant privacy, genetics and genomics data contain unique identifiers for each person. In 2008, the Genetic Information Discrimination Act (GINA) was signed into federal law, which protects against employer and health insurance discrimination due to personal genetic information [19]. Nurses involved with genetic biobanks need to become familiar with the scope and limitations of GINA to further their awareness of governances protecting biobank participants. GINA may potentially lessen the participant's fear of potential discrimination based on sharing their genetic information and increase their willingness to participate and donate biospecimens into biobanks for future research.

12.8 Conclusion

Researchers using biobanking resources are becoming increasingly widespread as scientists, including nurse scientists, recognize the need for large quantities of quality biospecimens and data. Consequently, the nursing profession has continued to broaden and evolve as biobanking expands. The perspectives the nursing profession brings to biobanking practices ensures that biobanks are designed with the protection and benefit of potential biobank participants and the contribution of pertinent nursing-relevant data meaningful to answering questions posed by nurse scientists. As genetic and genomic influences on healthcare multiply, the nursing profession is required to maintain professional competencies and address genetics and genomics and related ethical issues including those surrounding biobanking. The role of the nursing profession will become increasingly important as the breadth and scope of biobanking practices spread.

Educational Resources on Genetic Biobank Applications

The *Journal of Nursing Scholarship* Genomic Nursing Series Covers Important Perspectives to Prepare Nurses for the Translation of Genomics into Practice http://www.genome.gov/27552093
A Video Webinar of this Series is Available at http://www.genome.gov/27552312
A Series of Genetic/Genomic Articles by the National Human Genome Research Institute and National Cancer Institute for Nursing Educators http://www.genome.gov/27543639
Genetic Biobanking for Research Position Statement by the International Society of Nurses in Genetics (ISONG) http://www.isong.org/documents/BiobankingPositionStatementFINAL_February2014.pdf
2012 Best Practices for the Collection, Storage, Retrieval and Distribution of Biological Materials for Research from the International Society for Biological & Environmental Repositories https://isber.site-ym.com/?page=BPR
The Organization for Economic Co-operation & Development Guidelines for Human Biobanks and Genetic Research Database http://www.oecd.org/health/biotech/guidelinesforhumanbiobanksandgeneticresearchdatabaseshbgrds.htm
National Human Genome Research Institute National Institutes of Health Informed Consent Form Examples & Model Consent Language http://www.genome.gov/27526660
Genetic Resources of Particular Interest for Nurses by Cincinnati Children's Hospital http://www.cincinnatichildrens.org/education/clinical/nursing/genetics/default/

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A Data-Centric Strategy for Modern Biobanking

13

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Abstract

Biobanking has been in existence for many decades and over that time has developed significantly. Biobanking originated from a need to collect, store and make available biological samples for a range of research purposes. It has changed as the understanding of biological processes has increased and new sample handling techniques have been developed to ensure samples were fit-for-purpose.

As a result of these developments, modern biobanking is now facing two substantial new challenges. Firstly, new research methods such as next generation sequencing can generate datasets that are at an infinitely greater scale and resolution than previous methods. Secondly, as the understanding of diseases increases researchers require a far richer data set about the donors from which the sample originate.

To retain a sample-centric strategy in a research environment that is increasingly dictated by data will place a biobank at a significant disadvantage and even result in the samples collected going unused. As a result biobanking is required to change strategic focus from a sample dominated perspective to a data-centric strategy.

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Keywords
Biobanking • Big data • Biobanking strategy

13.1 Introduction

Biobanks collecting and storing human tissue samples to support research into disease and therapy have existed for several decades [1]. Over the last two decades, with the advent of more sophisticated tissue analysis technologies and the digitalization of health, the needs of bioscience researchers have changed rapidly. Many of the older biobanks, which have traditionally pursued a sample-centric strategy, have struggled to adapt to these new demands. This is particularly true with the data and extended metadata annotations associated with samples, which have increased massively in complexity from simple attributes such as date of collection, diagnosis and histology, to now include much more extensive patient phenotypic, prescription & dosing, co-morbidity, genotypic, proteomic, and other "omics" information. The continual rhythm of new technological development has become the new normal in bioscience research, and as ever more accurate and informative analysis tools are used, the need to add these new types of data to the samples will continue with it.

To remain relevant, and for their tissues to be useful in the future, biobanks must be able to continually adapt to these technological development and changing user demands. Historically such changes have been focused on the sample, such as the collection mechanisms, storage routines and quality measures. While continual improvement in these areas is also required, this is not the fundamental challenge for modern biobanks. Biobanking has reached an era where data and metadata extend far beyond the sample itself and now also include (1) the full clinical history of the donors across primary, secondary and tertiary care, (2) the position of the biobank and its samples within an integrated collection and research network and (3) the return of data about the samples generated by the researcher. These data are first used to identify or stratify the sample population, secondly to extend the reach of collections, particularly in rare diseases, by making samples more visible in a global network and thirdly to ensure that the samples' annotations are continually extended to give them maximum and continued benefit.

In this context, it is much more useful to think of modern biobanking as an informatics project supported by a high-quality, scientifically driven tissue collection and storage strategy, rather than as a high-quality tissue collection and storage strategy supported by an IT system. This is not a trivial semantic distinction – the factors that drive both the collectors' and researchers' ability to benefit from a biobank are all predicated on the consistency and quality of information describing the tissues that it contains and the ability of researchers to use this information to find the specific patients and tissues that they require accurately and quickly.

13.2 Sample-Centered Strategy and the Impact on Data

The sample-centered approach is clearly not unusual or unexpected, as the traditional role of a biobank has been to collect and supply samples. All biobanks have a strategy to collect and store samples for a particular identified need, which may have a disease focus or to sit alongside a clinical department to collect residual material from patients as they attend clinics. The sample-centered strategy places this sample collection at the core and the data is seen as an annotation/enhancement of the sample [2–4].

The act of collecting samples is not in itself a great challenge – by as early as 1999 over 300 million samples had been banked in the US from over 170 million cases [2]. A naïve sample-

centered approach, however, does cause issues for the data systems underpinning many biobanks, and this inevitably limits the usefulness of the samples in those collections. The International Society for Biological and Environmental Repositories (ISBER) Informatics Working Group sought to establish how well the biobank database systems were underpinning the functions of the biobank. Their survey suggested that there were no data or system issues linked to the collection of samples [3]. Half (4/8) of the features rated as the most important in relation to sample management were around the receiving, labeling, sending and tracking of samples and these features were all marked as 'at least adequate' [3]. Therefore, while debates will continue about the best processes to preserve samples in order to maintain high quality tissue, the best practice for collection and storage of samples is relatively well understood.

The remaining and fundamental challenges for most biobanks are downstream of the actual collection of the sample in the generation, handling and use of data associated with the samples. The common cause for these problems is the lack of a key objective during sample collection. Most biobanks now operate on a model where consent is obtained without specifying the exact research project [4]. Whilst in theory this allows samples to be used on multiple projects, the uncertainty caused by a lack of well-defined requirements filters incrementally into the three identified data challenges. The ISBER survey found that out of the features for sample management rated most important, the ability to dynamically configure annotation fields and Application Programming Interfaces (APIs) and systems integration were rated as least satisfactory [3]. These are all features that are directly relevant when attempting to integrate with or store new clinical data (challenge 1). All respondents rated their systems as 'Inadequate' in the ability to share and search data across departments and institutions (challenge 2) [3]. The ISBER survey, although mentioning the importance of such a feature, did not evaluate elements related to the return of data, such as from NGS sequencing, bioinformatics analysis or other 'omics studies.

It should be of no surprise that a lack of clarity around requirements leads to systems being incompletely designed and poorly perceived by researchers who spend a large amount of time annotating features that they then struggle to use when seeking to identify tissues for their research. Biobanking inherently operates within an uncertain and continually changing environment as the actual research use of samples or the techniques that will be available at that stage are often unknown at the point of collection. This is the consequence of the 'new normal' of continual technology adoption, which will only become more obvious in the future. To maintain their relevance and utility to biomedical researchers, biobanks must embrace a re-alignment in their strategy to allow them to operate successfully in an increasingly data intensive research environment.

13.3 A Data-Centric Strategy for Biobanks

When a biobank collects a sample it is always with one (potentially unstated) high level objective in mind, to enable the researcher to generate data from the sample and then use that data in combination with data held by the biobank or other institution to answer a research question. In this context, the role of the sample is a substrate to allow generation of data and annotations that is currently missing (such as protein or genetic information) and that researchers will use to select tissues that are relevant to their study. The sample itself is not only almost worthless without this data, but the collection and storage of tissues that cannot be found and used by researchers is both expensive and potentially unethical.

Biobanking 3.0 has been described as the way forward in biobanking with a shift away from samples as the center of the project and a much greater focus on data and the needs of the researcher [5]. This difference represents a fundamental reappraisal that goes to the heart of the purpose of biobanking and looks to prepare biobanks for the future needs for sample collection. As an example, when studies are completed that

identify areas requiring research [6, 7] the response should be to examine what data are required, from where and in what quantity in order to be able to answer the question. The ongoing utility of those samples in the wider research context should also be considered. Sample collection should only be initiated when the collection of the samples will enable all missing data to be generated and the full design of the research study to be conducted. Clearly there is no point starting sample collection if there is no prospect for the generation or integration of other data required (such as the treatment or lifestyle data). It is a misconception to believe a lack of samples prevents research – it is more often a lack of data that prevents research, and samples are a mechanism for generating data that is missing.

The UK Biobank [8] is perhaps one of the best examples of how this data-centric strategy can deliver continual scientific and clinical relevance over a period of decades. The UK Biobank has a core purpose of assisting researchers to determine the different risks of population cohorts developing a disease and how those risks interrelate. Prior to sample collection a comprehensive power analysis was undertaken in attempt to predict the number of cases and scale of data required in order to detect the causes of certain diseases [9]. It is important to note that the UK Biobank was not setup around any one research question but had a core purpose in which its sample collection and annotation policy could, with a high degree of confidence, guarantee certain types of questions could be answered. The setup of the bank is therefore similar to many operating today with a generic consenting model used in most biobanks. The decision around which samples to take was the most obvious difference to the normal approach, and the decision to collect a particular sample type was based on "the likely value of the additional information that would be made available by collecting some particular sample type" [9].

As the requirements of researchers have changed over the decade since its inception, the UK Biobank has been able to adapt and importantly has done this in the main by developing the scope and resolution of its current dataset, rather than by collecting more samples. Donors have been contacted to supply additional data or strategic linkages with other data resources to enrich the contextual detail stored in the UK Biobank database. The existing samples that were already collected have also been reanalysed to generate further standardized data, including a panel of 36 biomarkers [10] and genotyping data [11]. The outcome of this is that the researchers are presented with a unified data resource in which they can answer currently topical research questions. Over the most recent 12-month period all requests were in fact for data rather than samples.

The UK Biobank's data-centric approach benefits all three of the core data challenges. Data integration is improved via pro-active acquisitions and interoperability can be delivered as part of a timetabled investment rather than a series of costly *ad-hoc* responses to individual requests to the biobank. The networking and searching of biobanks becomes easier as the researcher can be presented with defined datasets for particular types of research, rather than trying to create an informatics system that must find the sub-group of samples suitable for a research question.

The largest potential benefit is the challenge of receiving data back from the researcher. For both the biobank and the researcher one of the key challenges is the heterogeneity of the data being generated and returned. The volume, variety and veracity of those potentially big data being returned and its likely actual usefulness all have a major impact on whether, where and how to store and use those data. The UK Biobank has been able to reduce this challenge by commissioning the use of the samples in order to generate the data that are most likely to be generated by researchers (in the first instance, genotyping and biomarkers). By supplementing the existing dataset with data from the samples including genotyping and biomarkers then the resource is infinity more valuable than if researchers had

independently created this data under a data return policy without controlled and standardized procedures.

A data-centric strategy delivers clarity of core-purpose that can feed into the design of the system and its technical choices. As demonstrated by the UK Biobank, and contained with the philosophy behind Biobanking 3.0 [5], biobanking does now operate within an increased culture of change. In this light new technological approaches to data management may be more appropriate, such as alternative data storage systems that seek to deliver the ability to store and analyse large datasets in a manner that can facilitate flexibility [12, 13]. In addition cloud storage can allow biobanks to have access to large, sustainable and scalable data storage as demonstrated by the 1,000 genome project [14]. However, the most appropriate technical solution should always be informed by the strategy of the biobank.

13.4 Conclusion

Biobanks face a challenge to meet the changing requirements of the researcher and these needs are increasingly related to creating suitable datasets. As such strategies that focus on the sample are no longer suitable and modern biobanking should be considered as an informatics project supported by a high-quality, scientifically driven tissue collection and storage strategy. Without such a data-centric strategy it is hard to envisage biobanks being able to remain relevant to modern research.

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The Importance of Quality Patient Advocacy to Biobanks: A Lay Perspective from Independent Cancer Patients Voice (ICPV), Based in the United Kingdom

14

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Abstract

Biobanking in the twentieth century will become of increasing importance in health research. Regulation and governance of biobanks must be open and transparent to ensure public trust and confidence and increase donation. Effective Lay Involvement all levels in biobank organisations should be standard practice helping ensure patient benefit remains the central aim and assisting the Promotion of Biobanks and Recruitment of Donors. Properly selected, educated and supported, they become valued members of the Biobank Team. This chapter is based on the work of Independent Cancer Patients' Voice (ICPV) in the UK and recognises that the National Health Service provides a framework which is not universal and neither is the model of patient advocacy which has been developed particularly in cancer research. However, although it has not been easy to find potential members for ICPV, nor to attract funding, we have earned the respect of our professional colleagues by our commitment in giving time and developing the skills necessary to provide effective involvement. These colleagues have enthusiastically mentored and supported us and have provided venues and tutoring for Educational Events. We are sure that patient advocates in other countries would welcome the opportunity for similar involvement and hope our experiences will be of interest.

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Keywords

Patient advocacy • Lay involvement in biobanking • Donors recruitment • Brain tumour tissue bank • Breast cancer campaign tissue bank • Patient-led consent

14.1 Introduction

Independent Cancer Patients' Voice (ICPV) is a patient led group founded 6 years ago to provide education, mentoring and support for people who, having been treated for cancer, wanted to add a more informed patient perspective to cancer research. The founders were lay members of the Breast Clinical Study Group (BCSG) of the National Cancer Research Institute who initially recruited other interested breast cancer patients. Some had undertaken the very effective Project LEAD Advocacy training offered by the National Breast Cancer Coalition in the USA and wanted similar education for patient advocates in the UK. Although initiated by breast cancer patients, ICPV is now a generic cancer patient group which is reflected in ICPV training events.

ICPV members wish to be active partners in research rather than just passive recipients of care. They recognise that effective input requires education as well as experience and, knowing that they cannot claim to be representative, they prefer the title of 'Patient Advocate'. Study days are held at academic centres across the UK with the enthusiastic support of professional colleagues who host and tutor these events. This reduces costs whilst increasing both collaboration and ICPV's geographic spread. These events started with a 1 day course in Leeds at the invitation of Professors Andy Hanby and Val Speirs and have expanded to some being run over 2 and 5 days. In 2013, Professor Louise Jones and Professor John Marshall at Barts Cancer Institute helped ICPV achieve their original aim – a 5 day residential course in biology – "Science for Advocates". This course was repeated in 2014 and is now annual with EU delegates registered to attend at 2015.

ICPV works with many other charities, academic organisations and government bodies but is independent – thus able to provide an informed and unfiltered patient perspective to cancer research and development of new treatments. Members of ICPV sit on many Trial Development, Management and Steering Groups, Executives and Boards. ICPV has stakeholder membership with the All Party Parliamentary Group on Cancer (APPGC), the National Institute for Health and Care Excellence (NICE), The Human Tissue Authority (HTA) and the Health Research Authority (HRA). It has been invaluable to have the support and encouragement of our professional colleagues together with the easy access to factual information about ethics, regulation and governance from Dr Janet Wisely and team (HRA) and Dr Shaun Griffin (HTA).

ICPV involvement in tissue banking has developed from involvement in cancer research and increases the need for more specialised education and mentoring. This involvement is within the framework of the UK National Health Service but could be applicable in services in other countries. The major aim of patient advocates actively involved with biobanking is to ensure that patient benefit remains the prime objective of any research using donated tissue and that this research is carried out to high level quality and ethical standards. Most donors would wish for maximum possible use of both tissue and related data for the benefit of future patients and want all data, including negative results, to be shared in order to increase knowledge and prevent duplication. However, it should not be assumed that all donors have no further interest in what happens with their tissue and current press interest is raising concerns. Open inclusion of patient advocates at all levels would help biobanks demonstrate transparency and

proper governance together with respect for the donors and their tissue, thus helping to retain public trust and confidence. However, some current projects do not currently involve patients and public and the huge potential benefit of exciting and innovative biobanking initiatives could be hampered by loss of public confidence. At the same time proposed changes in European Data Protection Regulations threatens the proper collection of health data for research. The collection, storage and use of data held in European biobanks is governed by national laws based on the EU Data Protection Directive. A new data protection regulation has been proposed by the EU to update the law, which includes exemptions for medical research with certain safeguards. However, the existing research exemption from consent would be restricted if the European Parliament adopts amendments to Articles 81 and 83 of the Data Protection Regulation. ICPV is a signatory to the Wellcome Joint Statement on this issue [1] and works closely with the National Cancer Intelligence Network (NCIN) on the ethical collection and use of health related data in cancer research and welcomes projects which safely link data across organisations.

14.2 Different Models: Specific Examples

The following pages show specific examples of different models of involvement in cancer biobanking by individual members of ICPV and will illustrate both the value of effective lay involvement and the potential for greater collaboration and innovation:

14.2.1 "Science for Education": A Participant Experience

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As a patient advocate and member of ICPV I was a student on this course, the first of its kind to be run in the UK. The course was a mixture of lectures and practical lab sessions. Topics covered included basic cell biology – how cells behave, grow and die, the nucleus, DNA, RNA, proteins, specialisation, signalling, oncogenes and tumour suppressor genes. Biomarkers, (very relevant to use of tissue samples), what they are, how they are used – to predict future risk/diagnose disease/prognostic outcomes in predicting the effectiveness of treatment and side effect risks. All of this was combined with daily sessions in the lab with the scientists – not just watching but as a hands on experience (Fig. 14.1): extracting DNA, treating cancer cell samples with toxic agents and measuring the effects with a biochemical test. The most memorable session for me was in the pathology lab as breast tissue was processed for diagnosis and tissue banking, following the process through the various stages. I am a breast cancer patient and I have had a mastectomy, I was so impressed at the way the pathologist and all the staff handled the tissue with such care and respect. How reassuring for patients the care shown to a part of you that you have consented to be used in research.

So was this simply an enjoyable week? I believe that research is an intricate part of quality health care and central within the NHS. As an advocate I am involved in partnering with researchers to ensure that research is ethical and of benefit to both patients and the NHS. The use of human tissue is an essential part of that research to help expand knowledge in the areas of how disease works, how disease can be prevented, diagnosed and treated. The opportunity to be part of the VOICE course gave me a level of understanding of the science involved and this in turn further equipped for effective lay involvement. The knowledge gained has enabled me to be more effective in reviewing research proposals and trial design; and the use of tissue samples with issues around consent for a specific trial; generic consent for future research. It has highlighted the importance of information given to people in relation to the donation of tissue, urine, blood and saliva samples, their preparation, storage and use; including access to their health data. The importance of trust when a patient gives that permission. There is the dual role of the patient

Fig. 14.1 ICPV members learning lab techniques

advocate in partnering with the pathologists and Biobank scientists and also engaging with the public.

14.2.2 Breast Cancer Campaign Tissue Bank

Mairead MacKenzie
Independent Cancer Patients' Voice (ICPV)
London, UK

The Breast Cancer Campaign Tissue Bank (BCCTB) opened in 2010 after the 2008 Gap Analysis [2] showed that research was being limited due to lack of available good quality tissue. From the very start Breast Cancer Campaign saw the importance of patient input and two patient advocates were involved in the early discussions and site visits to potential biobanks. Today there are five advocates taking active roles in the tissue bank; two sit on the management board and three on the tissue access committee. This means that no research project is approved or tissue released without the agreement of lay members. The lay reviewers do not need to be scientists, but have to have an awareness and understanding of research. Our key role is that if we don't see the patient benefit in a piece of research then we say so. Tissue is a valuable resource and donors need to be assured that their tissue is being used wisely. Breast Cancer Campaign took the extra step in involving patients at the very early stages of this project and we believe that lay involvement can only improve the standing of the bank. As BCCTB Chair Professor Alastair Thompson said *"Patient Advocates have kept us grounded in reality, have been very helpful with ethics and information sheet issues and have made us all realise that the standard practice of just throwing tissue away is a terrible waste of resources. They have also been a real pleasure to work with, have made good comments and often respond to e-mails better than professionals"*.

The Bank's data return policy was also driven by the patient advocates who were keen that the tissues donated were used to their maximum benefit. This has resulted in the first publication from Tissue Bank [3].

I have now been involved with the Tissue Bank for nearly 3 years and for the past year have been on the Tissue Access Committee. It is so important to have a patient view at this early stage of

research and I really believe that patient advocates can play a part in ensuring that all research proposed has the patient at its centre and that our precious tissue is used to its best effect. Any questions that I have posted to researchers have all been answered well with no feeling that I am asking the 'silly' question – although sometimes this can be the most pertinent. Patient advocates give the "public face" to tissue donation and can help promote the bank and the research that it provides.

The tissue bank advocates were interviewed for BCC newsletter in 2013 to help promote its use in the wider cancer community [4]. Patient advocates have also promoted PPI within Tissue Banks by having poster presentations at both the NCRI Conference 2013 and the San Antonio Breast Cancer Symposium 2013 [5].

Many of the researchers using the Tissue Bank rarely have any interaction with patients and Val Speirs, Professor of Experimental Pathology & Oncology, Leeds Institute of Cancer & Biology has said *"The phrase 'translational research' is now firmly embedded in the scientist's vocabulary but few have the opportunity to truly engage with patient advocates in the way that the people charged with the responsibility of running the BCCTB can. Having their views can really shape the future of translational search and help drive this forward, benefiting future generations of breast cancer patients."*(Note: since writing, 'Breast Cancer Campaign' and 'Breakthrough Breast Cancer' have merged to become 'Breast Cancer Now').

14.2.3 Brain Tumour Tissue Bank: The Brainstrust Proposal

Helen Bulbeck
Independent Cancer Patients' Voice (ICPV)
London, UK

To build a UK wide network of brain tumour tissue banks that will support a diverse range of research so that the prevention, diagnosis, and treatment of brain cancer are improved.

The need Brain cancer is an area of unmet clinical need. It is one of the most lethal human diseases; only 32 % of the 7000+ people diagnosed with primary brain cancer will be alive at the end of the first year following diagnosis and drops to 14 % at 5 years [6].

Despite these statistics, neuro-oncological research has been, until recently woefully underfunded. This has meant that there has been no structured research base for neuro-oncology and so it has become fragmented and uncoordinated. This is due mainly to the bureaucracy surrounding the use of human tissue, where tissue has been gathered first and ethical consent for use has followed. This has led to tissue banks being set up which are a closed resource to researchers.

Patient voice Significant changes are happening within the health sector which mean that patients have been able to engage with this project. Our community knows the importance of:

- Clinical need – brain cancer is being left behind. As survival rates for cancer improve, survival rates for brain cancer remain unchanged with the outcome of patients with high grade gliomas remaining poor with the median survival below 18 months [7].
- Empowerment – 'no decision about me, without me' is fundamental to the current political healthcare agenda. Patient empowerment and closer engagement with their care lies at the heart of this initiative.
- Stratified medicine – we are now treating the biology of cancer, rather than cancer. But to do this accurately and effectively large numbers of samples are needed. A particular challenge for the coming decade will be the increasing stratification of treatments and their tailoring to much smaller subsets of patients [8].
- Data is a valuable commodity – an ongoing modernisation of cancer registries, combined with new datasets now either mandated for collection or in the process of being mandated, is making a step change in the data available. Patients know this and are able to access their data.
- Increased patient awareness and understanding around the collection of tissue; patient

voice can drive the agenda. Seeking authorisation for tissue collection from patients makes it meaningful and creates space to talk. Individuals who are informed about biobanking are much more likely to participate and give broad consent [9].

By unlocking the potential value of collections of brain tumour tissue samples this project will facilitate many research studies. Shared reciprocity will be at the core – between patients, clinicians and researchers.

Willie Stewart, Consultant Neuropathologist at Southern General Hospital, Glasgow, says, 'one problem researchers in this field continually meet is a lack of tumour tissue for high quality research, yet there are vast resources of material in diagnostic laboratories throughout the country. This project paves the way for this invaluable material to be accessed to support high quality research projects' [10].

14.2.4 Confederation of Cancer Biobanks

Maggie Wilcox
Independent Cancer Patients' Voice (ICPV)
London, UK

The Confederation of Cancer Biobanks (CCB) in the UK has produced quality management and data standards. It is of interest that there was no mention of PPI in the report from an ISO workshop on International Standards for Biotechnology. However, the CCB is committed to effective lay involvement and included an ICPV member in each of the four working groups of the Harmonisation Project. The working groups covered (1) Public Engagement, Ethics and Consent (2) Sample quality (3) Biobank governance/IT (4) Quality Assurance [11].

A lay person chairs the Exec of CCB which also includes a member of ICPV – Both have been treated for cancer, are full members of the team and consider that their views are respected and genuinely valued by their professional colleagues. However, as people who have been involved in biobanking organisations for several years, they are still surprised at the lack of genuine lay involvement in research tissue banks and large cohort studies in the UK. Patients and the public as tissue donors are key stakeholders for any organisation or project that is collecting human samples for research and, without their support and trust, there would be no samples. Some organisations rely on the fact that many research participants give their consent without actually being aware of what research may be undertaken or whether the sample will ever actually be used. PPI in the work of the biobank provides reassurance that research is for genuine patient benefit and that ethical considerations and high quality standards are maintained. Indeed, biobanks and large epidemiological studies could risk losing public trust by not engaging and involving lay advocates. CCB member biobanks who have involved patients can illustrate the benefits of embedding this activity throughout the organisation of the biobank; a position that the NCRI and Confederation of Biobanks are fully supportive of and recommend in their biobanking standard [11].

Wales Cancer Bank (WCB) and Breast Cancer Campaign Tissue Bank (BCCTB) are members of the CCB with established PPI at all levels. In the early days of planning, patients and lay people were involved in discussions about the scope and proposals for developing these tissue banks. In Wales patients were part of the Steering Group which developed the bid for funding and establishment of the tissue bank. They also provided key input into the participant information sheet and consent forms as well as advising on the Ethics committee application and when to approach patients regarding tissue donation. Dr Alison Parry-Jones, Manager of Wales Cancer Bank considers that the input on this latter aspect was vital. "We would have had much more complicated processes and caused ourselves issues if we hadn't involved our Lay Liaison Group. We had preconceived ideas of how sensitive patients might be at the time of being diagnosed with cancer and were told very firmly by our lay colleagues that we should just go ahead and approach them. If it's not a good time they'll tell us but they are people and they're not made of glass."

This challenging of pre-conceptions was also apparent during the establishment of the BCCTB

who included two patients on their Management Board when the applications to host the bank were being reviewed. Alastair Thompson, Chair of BCCTB highlights, "As researchers and clinicians we saw this as a competitive process but rather than looking for a single winner, our lay colleagues challenged us to rethink the process and said – 'there are several good bids – why can't they work together to create a virtual bank?' It's fair to say the altruism of patients donating tissues to the bank is enhanced by those patient advocates guiding the workings of the bank."

Dr Bridget Wilkins, Lead Pathologist at NCRI, is a member of the CCB Exec and facilitated ICPV collaboration with Trainee Pathologists to use questionnaire responses from public, patients and clinicians to inform the potential production of a "Lay Guide to Tissue Donation"

This project is continuing at present.

14.2.5 Local Cancer Partnership Research Group Event: October 2013

Robert Flavel
KSS Cancer Partnership Research Group
London, UK

The Surrey, West Sussex and Hampshire Cancer Partnership Research Group (SWSH CPRG)[1] comprises a group of patients, carers and healthcare professionals interested in cancer research. The group was founded in 2004 under the auspices of Macmillan and is based in Guildford. The group is now part of the Kent, Surrey and Sussex Clinical Research Network.

The main aim of the group is "to involve patients and carers in cancer research, ensuring that it is easily understood and accessible to all". The group feels so passionately about Tissue Banking that it devoted its Annual Educational Research Event to the subject.

The event was held at the Royal Surrey County Hospital (RSCH) in the Post-Graduate Education Centre. Dr Albert Edwards, Prostate Brachytherapy Research Fellow at RSCH and member of the CPRG, chaired the event, encouraging lively discussion and interaction between the audience and the speakers. The guest speakers were Dr. Agnieszka Michael, Clinical Lead for then SWSH CRN and Director of the Tissue Bank at the University of Surrey, Dr. Balwir Matharoo-Ball, Operations Manager for Nottingham Health Science Biobank and Dr. Bridget Wilkins, NCRI lead for Pathology and executive member of UKCCB.

The many topics covered by the presenters included: How to set up a tissue bank and the resultant data protection implications; Using lay people to obtain consent from patients for donations to the tissue bank; Using NHS stored biopsy and tumour samples as the basis for a tissue bank.

The event was attended by approximately 60 people including members of the public, healthcare professionals (GP's, oncology clinicians, specialist and general nurses, pharmacists, pathologists), research staff and research data managers.

Feedback from the audience was very positive with the following quotation being a typical example:

> I have not really paid too much attention to tissue banks before, but I found your event most interesting. With the steady move towards gene therapy, cell treatments and immunotherapy...the 'bank deposits' will have a great influence on research and hopefully treatment of cancer.

What is also very pleasing is that one of the pathologists who attended the event is leading the setting up of a Tissue Bank at RSCH. Perhaps a good example of PPI helping cancer research.

14.2.6 A New and Innovative Patient-Led Consent Pathway for the Nottingham Health Science Biobank (NHSB), Nottingham, UK

Hilary Stobart
Independent Cancer Patients' Voice (ICPV)
London, UK

Consent to donate tissue and data is an expression of partnership and goodwill between donors and a biobank. Research has shown that consent

[1] Since April 2014 the CPRG has been a part of the Kent, Surrey and Sussex Local Clinical Research Network (KSS CRN).

rates are typically high if patients are made aware of the opportunity to donate tissue and samples, and informative and accessible, but also sensitive and user-friendly consent pathways are key to this. In 2011 the team at Nottingham Health Science Biobank set out to improve its processes around patient consent to donate tissue and samples, and began working with their Patient and Public Involvement Advisory Group to develop improved methods.

The Nottingham Health Science Biobank is an NHS Trust led initiative which along with its related bioinformatics strategy creates a platform for translational and clinical research. Patients are invited to consider donating surplus tissue that arises from tests and treatment, along with blood and urine samples, to create a single, centralised, quality assured, biofluid and tissue resource to underpin translational studies and to add value to clinical trials.

I was privileged to be part of the initial PPI Group, when it was suggested that the consent process could be driven and delivered by patients and volunteers. NHSB went on to design a comprehensive consent training package, including presentation, role-plays, hand-holding, shadowing observation, competencies and final sign-offs. Five of us volunteered to be involved and received a full induction by the hospital Trust, and were offered honorary contracts. We were, of course, required to complete all required safeguarding checks and then undertook the consent training package. Since we started in 2011 we have taken on responsibility for taking consent in all of the out-patient clinics in a busy regional centre breast unit, and have spoken to several thousand new and follow-up patients between us.

Patients are sent copies of the information leaflets and consent forms for the biobank with their initial appointment letters, and are offered the opportunity to discuss further, ahead of their appointment with the healthcare team. The new pathway and role of the volunteers has had excellent feedback from both patients and volunteers taking consent and has led to increases in consent rates. I, personally, was initially surprised at how willing almost everyone is to have a conversation on the subject of donation, whether or not they go on to choose to donate themselves. In fact one of the values of the conversation with patients is an opportunity to increase awareness generally of the need for research and the role of donated tissue and data. Even those who choose to decline or wish to consider their options at a later date are usually appreciative of the chance to consider the issues.

An advantage of the new approach is that the volunteers and patients generally have more time to discuss the issues arising than would be possible for clinicians in the midst of health-care appointments. A further added benefit of the process is the separation of the consent process from the discussion with doctors. At Nottingham consent is both generic and enduring and this separation helps patients to consider this long-term use of their tissue and data away from the immediate pressure of current decisions about their health-care.

I am pleased to be involved in the next roll-out of the process at Nottingham where those who already take consent will be involved in training new patient advocates. It is good to know that there has been much interest in the model and requests are often received from other centres for the training and consent packages.

14.3 Lay Involvement in Action: What Is Possible?

ICPV has presented papers on the value of Lay Involvement in Tissue Banking at Patient Advocacy Conferences/Meetings in Cape Town, Milan and Bucharest, the European Cancer Organisation Conference in Amsterdam, the San Antonio Breast Cancer Symposium as well as here in the UK. When NCRI invited ICPV to host a parallel session on Tissue Banking at their annual conference in 2013, the remit was to include young researcher, European and lay perspectives. This was achieved by input from the European Organisation for Research and Treatment of Cancer (EORTC) and scientists from University College London & the University of Manchester together with 2 lay speakers under the Title of “The Issues about Tissues”. A Fringe

Fig. 14.2 ICPV Group after a meeting at House of Commons

meeting about the need for human tissue in cancer research was hosted by ICPV at the annual conference of the All Party Parliamentary Group on Cancer in 2013 (Fig. 14.2). Chaired by Baroness Diana Warwick from the Human Tissue Authority, panel members included Helen Bulbeck (Brainstrust), Victoria Chico (School of Law, Sheffield), Prof. Charles Swanton (UCL), and Mathew Cooke (ICPV).

Involvement of patient advocates is valuable in many aspects of biobanking and should be integral at all levels from management boards to tissue access committees to public engagement activities. However, to do this properly requires selection and training of both patient advocates and the professional staff and ICPV recommends that experienced patient advocates should be part of this process. Some biobanking organisations are still avoiding the inclusion of lay members other than as donors of tissue. This does not, currently, cause a barrier to recruitment as the British public still has great trust in the NHS so that anything badged as NHS is generally accepted as safe. However, with recent controversies regarding the use of health related data, supply of DNA data to Europe and the implementation of "any suitable provider", we consider that public trust and confidence is being put at risk. ICPV was invited to contribute to the Parliamentary Office of Science and Technology briefing document on biobanks which confirms this as a potential risk for biobanking [12].

Biobanks which can demonstrate active and effective lay involvement at all levels can earn and retain the trust and confidence of donors and their relatives and this could lead to increased participation. Patient advocates can work with biobanks to increase public awareness of the need for tissue and related data in health research and ICPV considers that donation of tissue should become as acceptable as blood donation. Researchers in London have shown that where there has been previous experience of biopsy or personal family experience of breast cancer, there is a much greater interest in donating tissue for research [13]. It is likely that wider publication of the work of biobanks will increase interest of the general public and ICPV would like to see greater public engagement as well as patient involvement in biobanks –e.g. giving donors of tissue the

choice of further involvement by receiving newsletters giving updates about research using donated tissue, fundraising, publicity, ethical oversight and governance. However, whilst interested donors should feel they are valued as partners/shareholders in "their" biobank, the views of those opting out of further contact should be respected. During a recent effective campaign in USA, the Susan G. Komen Foundation was able to recruit healthy women to donate normal breast tissue for use in breast cancer research – this may become possible in other countries but, for most, the aim should be that donation of tissue from tumours is accepted as standard clinical practice.

Current advances in knowledge of the biology of cancer cells, including metastatic cancer, needs access to samples from different areas of a tumour and then from metastases. This raises ethical, sensitivity and patient safety issues but, when effectively explained to potential donors, is usually acceptable in practice. Patient advocates can help scientists and clinicians explain the need for such tissue to patients after diagnosis of secondary disease. Many doctors and nurses are protective of patients in their care, especially at times of great stress such as hearing that metastases have been found, and will be reluctant to add further stress. However, this creates barriers to important research which may not help this patient but would enable this patient to help future patients – this altruistic donation can give some comfort by adding a positive aspect to a very negative experience. By not discussing the possibility of donation, the clinician is actually preventing patient choice when the attitude of most patients will be "Why Not?" Obviously, the site of some metastases makes collection difficult but ICPV suggests that these patients should be given the option of post-mortem donation. Careful explanation and much greater public awareness of the need for such tissue is needed to make the latter an acceptable practice for clinicians, patients and their families. At the same time there needs to be discussions with pathologists, GPs and palliative care providers to have policies and procedures in place to ensure safe, timely and effective collection can be available when needed. Effective communication with potential donors and their families will also prevent unrealistic expectations and possible distress if particular tissue is not required. The GIFT Bank, run by Aidan Hindley in Leeds, is an excellent example of a bespoke tissue service for researchers using efficient and empathetic organisation of donation of tissue to be collected post mortem (www.gift.leeds.ac.uk).

ICPV has very recently joined a working group chaired by Dr James Flanagan at Imperial College London regarding an innovative collection and use of human tissue. This group is collecting donated breast milk to harvest epithelial breast tissue cells which they will use to increase their understanding of the mechanisms driving epigenetic variation which will improve breast cancer risk prediction and enable better targeting of preventative treatments. This has great potential for patient benefit but, by giving healthy young women the opportunity to donate excess breast milk, this is also an excellent opportunity to raise public awareness of the need for tissue in cancer research.

14.4 Feedback of Findings to Donors of Tissue for Research

There is variation in practice regarding the feedback of both incidental and research findings. This does not reflect donor choice but is usually governed by individual biobank policy. However, with the increasing role of biobanks and genomics in health research, there is a growing debate on the subject of donors' rights to receive feedback and how this should be managed. Some tissue provided is non-identifiable but where related follow-up health data is needed the tissue has to have some identifier.

In 2012 ICPV invited views from members and other patient groups as to whether feedback should be offered. Comments received generally fitted with two quotes from Genetics in Medicine/ Special Article "Managing incidental findings & research results in genomic research involving biobanks and archived data sets" [14].

- Kohane et al. [15]
- Offering discoveries back to individual research participants allows them to be "partners in research rather than passive, disenfranchised purveyors of biomaterials and data"
- CIOMS 1990s [16]
- International Ethical Guidelines for biomedical research involving human subjects has provided that "individual subjects will be informed of any finding that relates to their particular health status"

Most who responded felt it was their right to be offered feedback and that it was ethically wrong to withhold this. Some expressed strong belief that this was not a decision which researchers should be taking on behalf of donors – which was seen as patronising or paternalistic.

However, the majority qualified their views by saying that some advice/counselling would be needed alongside receiving the findings – together with appropriate referral for further investigations and/or treatment. Some said they were not so sure that they would want to know that they were at high risk of developing certain conditions – e.g. dementia – whilst others said this would be important to them as it would enable them to make proper provision whilst they were able to do this effectively.

It appeared that some were not fully aware of the possible implications of receiving feedback, for themselves and/or for their families, nor of the cost implications for the NHS in providing the information and counselling which would be needed. The general public do not realise that clinicians do not know themselves what the vast majority of coding alterations in the human genome mean so feeding this information back to patients could be seen as irresponsible and could cause unnecessary distress. Caution therefore needs to be exercised together with collaborative efforts to increase public understanding of the implications of feedback.

The Wellcome Trust then published a report which showed overwhelming interest in feedback of health-related findings to research participants- particularly when serious and treatable [17].

In the US, a consensus statement from the National Institutes of Health (NIH) with specific regard to biobank research says: findings that are (i) analytically valid, (ii) belie an established and substantial risk and (iii) are clinically actionable should be returned to participants, where such consent has been given [14].

Feedback of findings is still being debated by professionals and interested lay people and provision of feedback is still very variable in practice. Much more open discussion between biobanks and potential donors is needed to establish guidelines which are acceptable to donors, researchers and biobanks.

14.5 Validations from Researchers

"Patient Advocates have kept us grounded in reality, have been very helpful with ethics and information sheet issues and have made us all realise that the standard practice of just throwing tissue away is a terrible waste of resources. They have also been a real pleasure to work with, have made good comments and often respond to e-mails better than professionals."

Professor Alastair Thompson, Professor of Surgery, MD Anderson Cancer Center, Houston Texas and FORMER Chair of NCRI Breast Clinical Studies Group

"Patients challenge the somewhat paternalistic attitudes of the medical profession which impacts on what we consider to be acceptable to patients. This has the very positive effect of promoting much more open discussion of how patients can be approached to discuss donation to the tissue bank – and, in particular, has guided (and been very thought provoking) on how we might address more sensitive issues such as collection of metastatic lesions. Of equal benefit has been the opportunity to discuss in detail the huge value of tissue banking to the research community through which patient advocates actually become powerful spokesmen for tissue donation which is invaluable."

Louise J Jones, Professor of Breast Pathology, Barts Cancer Institute – a Cancer Research UK Centre of Excellence

"It is vital that patients have a say in how the Breast Cancer Campaign Tissue Bank works. The patient advocates involved with the Breast Cancer Campaign Tissue Bank make sure the patient perspective is considered in all key decisions."

Dr Lisa Wilde, Former Director of Research, Breast Cancer Campaign

"ICPV have been an invaluable to provide patients' perspective to our translational research program to understand cancer evolution through longitudinal cohort studies such as TRACERx. From trial concept development, through to protocol writing and regulatory submission, ICPV have provided invaluable advice at every step of the process. Their network and attention to detail is unparalleled. Their ability to canvas opinion during protocol development has helped us adapt to the needs of patients rapidly, accelerating the approval process and hastening trial recruitment. I look forward to further collaborations with ICPV during the course of TRACERx and other studies we are planning [18]."

Professor Charles Swanton, UCL Cancer Institute, Cancer Research UK London Research Institute and The Francis Crick Institute, London

"The involvement of lay people in collections of tissue samples for research has been critical in many ways but particularly in allowing professionals to feel confident about what can be reasonably asked of patients in their research partnership with them. Lay advice has been and remains very important to us in our trials of presurgical treatments of primary breast cancer; without this it is highly unlikely that these trials could have been successful."

Professor Mitch Dowsett, Breakthrough Research Centre, Royal Marsden Hospital

"Current progress in research, such as the ICGC/TCGA cancer genome projects, are founded on donations of samples from interested, altruistic patients across the world. Novel approaches to molecular diagnosis of disease will require continued engagement of donors across disease sites if we are to see progress in stratified medicine. There remain tensions between research and patient care, between access to samples and privacy for patients, between the rights of the donor and the responsibility of the recipient – be they medical practitioners or researchers. No-one is more able to speak to these issues than the donors themselves, and no-ones' voice should be more prominent than theirs."

Professor John Bartlett, Provincial PI Ontario Tumour Bank, Member of CTRNet,

14.6 Conclusions

Lay involvement in Biobanks should not be a "tick the box exercise" to meet current NHS or other organisations' expectations but should be integral at all stages of development, at all levels and in all activities. This involvement should be honest, effective, and evident in any biobank literature, as this will increase public confidence. However, members of the public who take on this role should commit to giving time for self-development and education to empower them as informed, realistic and effective lay members of the Biobank. Mutual respect and effective collaboration between lay members and professionals is essential for biobanks to achieve their potential value in health research and thus for future patient benefit. This process of involvement is a learning opportunity for both the lay members and the professionals involved. The lay members are able to increase their understanding of cancer biology and biobanking processes. At the same time, professionals are able to improve their understanding of the concerns and needs of potential donors. Most cancer patients are unaware of the intricate work which is done by pathology departments and which is essential for them to receive optimal treatment for their particular tumour. Lay involvement can also provide the vital connection between the bank and the potential donors. The role of ambassador and advocate is one that many patients take on when they become involved in research and tissue banking is no different. Raising awareness of research using tissue samples is becoming even more important with the development of genomic

technologies and the need for samples to be collected at multiple timepoints to monitor the progression of diseases like cancer. The consent and willingness of patients and the public to participate in this research will be vital and their involvement will help ensure that the trust and transparency, which is needed, can be maintained.

From the patients' perspective this can be a very rewarding activity. It is fascinating and exciting to learn about the science being carried out with the samples and some donors really do want to know how they are helping progress in medical research. Many biobanks don't send a newsletter to their donors or have information on their website about the research being carried out. How will they continue to be sustainable if they don't capitalise on the additional resource that their donors can provide in becoming advocates for them?

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