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COMMON MINIMUM TECHNICAL STANDARDS AND PROTOCOLS FOR BIOLOGICAL RESOURCE CENTRES DEDICATED TO CANCER RESEARCH

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The report was compiled and edited by an IARC secretariat composed of Elodie Caboux, Amelie Plymoth and Pierre Hainaut. In publications, it should be referred to as: COMMON MINIMUM TECHNICAL STANDARDS AND PROTOCOLS FOR BIOLOGICAL RESOURCE CENTRES DEDICATED TO CANCER RESEARCH, WorkGroup Report 2, Caboux E, Plymoth A and Hainaut P, Editors, IARC, 2007.

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PREAMBLE

Biological Resource Centres (BRCs), as defined by the Organisation for Economic Co-operation and Development (OECD), are service providers and repositories of living cells, as well as genomes of organisms, of archived cells and tissues, and of information relating to these materials (Organisation for Economic Co-operation and Development and Directorate for Science, 2001). Collection, annotation and use of human biospecimens are essential activities of cancer research. These processes are becoming critical in allowing patient access to molecular-based diagnosis and prognosis. BRCs need to comply with strict ethical and technical requirements and may be subject to regulation and/or legislation at various levels (institutional, local or national governmental, international). The aim of this document is not to replace such regulations. It is limited to tissue collections not needed for diagnosis or transplantation. The present document focusses on developing recommendations towards common minimum technical standards in order to stimulate the creation, development and networking of BRCs at the international level, and, through their use, enhance and improve cancer research worldwide. Ethical, legal and regulatory standards and recommendations will be addressed in a separate document, with the objective of promoting common standards while taking into account cultural and social differences among countries and states.

The International Agency for Research on Cancer (IARC) is working with a network of Directors of National Cancer Centres and organisations worldwide to develop this initiative. It aims to act as a catalyst and to provide a framework for the development and coordination of BRCs in all geographical regions. BRCs are essential for implementing a global description of cancer diseases based on molecular criteria. Developing such a description is key to further progress in descriptive epidemiology of cancer, understanding its causes, and working out novel preventive and cancer management strategies, the principal missions of IARC. Structuring, developing and harmonizing the calculation of the cancer-predictive value of biomarkers by means of prospective cohort studies is crucial to establishing the statistical parameters for cancer screening, and to give insights into cancer-causative mechanisms. This initiative is of particular importance given the central place of BRCs as a structural link between clinical practice, cancer registration and biological research aimed at understanding mechanisms and developing new treatments. A major priority in this initiative is the promotion of consensual standards acceptable and applicable in countries of different cultural, social and economical backgrounds, including training and the transfer of know-how, experience and technologies to centres located in low-resource countries, endowing them with a better capacity to develop research.

All researchers involved in biobanking activities are invited to consider and to adopt these recommendations which are applicable to different types of studies (prospective, retrospective or small collections).

This document has been developed in a stepwise manner, based on the recommendation of a group of experts of National Cancer Research Centres that met at the National Cancer Institute, Singapore, 19-21 April 2006. The first draft of this document was released on the IARC website (www.iarc.fr/goodscientificpractice.php) in December 2006 for a period of public consultation. The comments, remarks and criticisms contributed by many colleagues around the world have been taken into account in developing this first release of “Common Minimum Technical Standards and Protocols”.

The recommendations and protocols are largely based on guidelines, procedures and documentation on biorepositories developed by a number of working groups, institutions, regulatory bodies and organisations throughout the world. Table 1 presents a list of the main sources of information used for developing the recommendations presented in this document. Whenever appropriate, the document indicates references and links to more extensive documentation and protocols.

Table 1: Guidelines, procedures and documentation on biorepositories

Title	Authors/Origin	Link
Tissue Banking for Biomedical Research	Dr Oi Lian Kon; National Cancer Centre/Singapore	http://www.bioethics-singapore.org/resources/pdf/AppendixB-Dr%20Kon.pdf
Biorepository Protocols	Australian Biospecimen Network, (ABN)/ Australia	http://www.abrn.net/pdf/ABN_SOPs_Review_Mar06_final.pdf
European Human Frozen Tumor Tissue Bank TUBAFROST	TUBAFROST/The Netherlands	http://www.tubafrost.org/research/moreinfo/deliverables/TUBAFROST%20Deliverable%204.1.pdf
Human Tissue and Biological Samples for Use in Research: Operational and Ethical Guidelines	MRC/UK	www.mrc.ac.uk/consumption/idcplg?IdcService=GET_FILE&dID=9051&dDocName=MRC002420&allowInterrupt=1
Best Practices for Repositories I: Collection, Storage, and Retrieval of Human Biological Materials for Research	ISBER/USA	http://ehs.sph.berkeley.edu/Holland/Biorep/BestPractices2005.3.5.pdf
National Cancer Institute: Best Practices for Biospecimen Resources	NCI/USA	http://biospecimens.cancer.gov/global/pdfs/NCI_Best_Practices_060507.pdf
Transport of Infectious Substances	United Nations Economic Commission for Europe,UNECE/International	http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2005_22r%20.pdf
UN Recommendations on the Transport of Dangerous Goods. Model Regulations.	WHO/International	http://www.unece.org/trans/danger/publi/unrec/rev13/13files_e.html
A Cold Greeting: an Introduction to Cryobiology	Bioteach/International	http://www.bioteach.ubc.ca/Bioengineering/AColdGreeting/
Specimen Collection, Preparation, and Handling	Labcorp/International	http://www.labcorp.com/datasets/labcorp/html/frontm_group/frontm/section/speccol.htm
Recommendation Rec(2006)4 of the Committee of Ministers to Member States on Research on Biological Materials of Human Origin	Council of Europe Committee of Ministers	https://wcd.coe.int/ViewDoc.jsp?id=977859&BackColorInternet=9999CC&BackColorIntranet=FFBB55&BackColorLogged=FFAC75
The standard MM13-A "Collection, Transport, Preparation and Storage of Specimens for Molecular methods: Approved Guideline"	Clinical and Laboratory Standards Institute	http://www.clsi.org/source/orders/index.cfm?section=Shop&ETask=1&Task=1&SEARCH_TYPE=FIND&FindIn=0&FindSpec=MM13&x=9&y=2
The Human Proteome Organisation (HUPO)	Human Proteome	http://www.hupo.org/
Case Studies of Existing Human Tissue Repositories: "Best practices" of a Biospecimen Resource for the Genomic and Proteomic Era	RAND corporation	http://www.rand.org/pubs/monographs/2004/RAND_MG120.pdf
Biological Resource Centres: underpinning the future of life sciences and biotechnology	OECD/International	http://wdcm.nig.ac.jp/brc.pdf
OECD Best Practice Guidelines for Biological Resource Centres	OECD/International	http://www.wfcc.nig.ac.jp/Documents/OECD.pdf

DEFINITIONS

In this document, the term **Biological Resource Centre** and the acronym **BRC** are used to identify the whole range of biorepositories and related services dedicated to cancer research that are based on collections of human specimens. This definition is similar to the one developed by the Organisation for Economic Co-operation and Development (OECD), but in the present document there is a focus on biorepositories and services involving collections of human specimens for cancer research. The definition of a BRC not only involves the infrastructure for collection, archival and storage of biospecimens and data, but also the procedures and services for informing individuals who are approached to participate in a study, obtaining their consent, collecting and processing specimens for secure long-term storage, accessing and retrieving specimens appropriate for analysis, processing for preparation of biomaterials (e.g. DNA, RNA, proteins), for quality control, packaging and shipping specimens, etc.

Many types of BRCs are relevant to cancer research. They include, among others, biorepositories of tumour tissues obtained in the course of normal clinical procedures (often called tumour banks), specialised collections developed in the context of clinical trials, mechanistic studies, diagnostic or prognostic studies, collections of specimens developed in epidemiological studies, and biomarker studies biomaterials (tissues, blood, cell lines, DNA, proteins, etc.) derived from individuals with a history of hereditary/familial cancer.

The term **Institution** is used to identify the body responsible for specimen collection and archival that commits itself to the development, management and long-term maintenance of a BRC. While the organisational nature of such institutions may vary widely, they are primarily clinical cancer centres, academic medical centres, research institutes closely associated with clinical centres, or central organisations dedicated to the management of BRCs.

Several terms can define the person who is the source of the biospecimen. It is important to make the distinction between these definitions. For example, a **donor** is a person who donates or gives an organ, blood or blood products to another person, while a **patient** is someone who receives medical attention, care or treatment. An **individual** means the person who is the subject of protected health information, and a **participant** is a person who takes part in a trial. Participants usually must meet certain eligibility criteria.

Roles of biological resource centres in cancer research

BRCs play a number of critical roles in all aspects of biological research. The role of BRCs in biological research in general, and their impact on medical, societal and economical issues has been extensively discussed in a recent report of the OECD (OECD, 2004). This paragraph addresses the importance, justification and sustainability of developing BRCs for cancer research.

Importance of BRCs

BRCs are critical for cancer research

Human biological specimens have been used for many decades for translational purposes in cancer research, in particular for testing hypotheses and assessing biomarkers identified in experimental studies. The advent of novel technologies opens unprecedented opportunities to assess the status of the human genome, its expression, the complex networks of interactions between biomolecules and the functional consequences of their alterations. Therefore, studies on human specimens are also becoming critical in the process of discovering new mechanisms involved in causing cancer or in determining its progression, resistance/response to treatment and clinical outcome. BRCs are the foundation of three rapidly expanding domains of biomedical sciences: molecular and genetic epidemiology (aimed at assessing the genetic and environmental basis of cancer causation in the general population as well as in families), molecular pathology (aimed at developing molecular-based classification and diagnosis procedures for cancer diseases), and pharmacogenomics/pharmacoproteomics (understanding the correlation between an individual patient's genotype/phenotype and response to drug treatment).

BRCs are important for developing personalised medicine

Collecting and analyzing biological specimens is a necessary procedure for pathology-based diagnosis and is also a mechanism for allowing patients to benefit from the applications of molecular cancer research.

Close involvement of the pathology department at collecting centres is essential to facilitate the use of banked fresh frozen samples in diagnostic procedures. In the future, correct assessment of patient status and therapeutic needs may require the determination of a number of molecular parameters and will require systematic preservation of frozen biospecimens or derived biomaterial. With the continuing improvement of survival after therapy, performing such molecular-based assessment may become a systematic requirement not only at diagnosis but also at different stages of patient follow-up. While the present document specifically deals with biorepositories for research, it is recognised that developing BRCs may rapidly become part of recommended, if not mandatory, medical practice. Thus, gathering know-how and procedures for collecting, storing and analyzing human specimens is a major contribution to the development of biomedical practice worldwide. Therefore it is recommended that comprehensive cancer centres and academic medical centres have well-organized BRCs that actively cooperate in national and international networks of tissue banks.

BRCs have an impact on biotechnological and medical innovation

In the chain from laboratory discovery to medical application, BRCs have a key contribution to life science research and development (R&D). Progress in medicine is dependent upon innovation, development and translation of laboratory findings into clinical practice. Access to human biological specimens is often a prerequisite for such R&D advances. Thus, development of high-quality BRCs has the potential to accelerate and facilitate this translational process.

Importance of networking and exchanges between BRCs

Cancer is a global disease, the understanding and management of which requires comparisons between disease patterns in different parts of the world. In addition, studies on many rare forms of cancer are limited by the difficulty in recruiting a sufficient number of cases within any single collection centre. Networking between BRCs implies a multi-directional flow of information, know-how and biological materials between centres in different parts of the world, and requires that all laboratories adopt common technical standards for specimen collection, storage, annotation and data management. BRCs have an important role in facilitating such exchanges and in providing logistics and infrastructure for multi-centre research projects (epidemiological studies as well as clinical trials). It is recommended that the institution develop tools to enable up-to-date, anonymous information retrieval of clinical annotation on individuals, and set up communications between departments of (e.g.) oncology, surgery, pathology and clinical chemistry. BRCs need networking with the global scientific community in cancer research.

Issues in developing BRCs and using banked specimens

Developing and using BRCs requires the active involvement of many actors at different levels (national policy makers, institutional administrators, epidemiologists, pathologists, surgeons, clinicians, bioinformaticians, laboratory scientists) and has complex ethical and legal implications. The perception of these issues and the way they are regulated and managed varies according to legislative, cultural and economical contexts. This paragraph does not intend to provide general answers to these questions, but to put into perspective some important challenges associated to the collection, storage and use in research of human biospecimens. The Helsinki Declaration provides the general framework in which these questions should be addressed (1964).

Firstly, the rights of the individuals whose tissues or biological specimens are to be included in the BRC should be strictly considered and protected. Crucial aspects in this process are the development of appropriate methods to obtain informed consent according to the local standards where the definition of protocols are fully compatible with the three basic ethical requirements of autonomy, beneficence (non-malevolence) and justice (The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, 1979). In this process, it is critical that individuals receive accurate information regarding the potential use of their specimens in large national and international studies as well as in collaborative studies involving third parties such as industrial or commercial partners.

Secondly, the institutions that organize and oversee BRCs have the responsibility of protecting individual information and data, to guarantee safe and adequate long-term preservation of banked specimens, to inform, train and protect staff involved in specimen management, to ensure biological and environmental safety, and to make collections accessible and available under defined conditions for research purposes.

Thirdly, the scientists who wish to use banked biospecimens for research purposes must submit their research proposals and protocols to appropriate scientific and ethical review. As custodians of the biospecimens, institutions have the duty to take into account the non-renewable nature of the specimens in making priorities for scientific use. In requesting specimens from a biobank, scientists should develop detailed power calculations and provide pilot data to ensure the optimal use of biological resources. Distribution of specimens for research should be done within clear transfer agreements. Such agreements may include return of data and leftover materials

to the BRCs. They should also make provisions for users to contribute to the economical sustainability of the BRCs (see below), and should also acknowledge the rights of the BRCs and its scientific contributors to intellectual property derived from research performed using specimens made available by the BRC.

Principles for sustainable BRCs

Developing and sustaining a BRC has a high initial cost as well as running financial cost and can strain economically underprivileged institutions. These constraints are a significant obstacle to developing BRCs in middle- or low-resource countries. Lifting these obstacles in these countries requires a significant, international solidarity effort. The public sector (local, national governments, international bodies and organisations) has a responsibility for contributing to the funding of the baseline infrastructure of BRCs. On the other hand, the responsibility for development and maintenance of sustainable and usable specimen collections lies primarily with the institutions. Institutions should make provisions towards maintenance of infrastructure, equipment, running costs as well as data management systems. In addition, users of BRCs should contribute to the general financial and structural sustainability of BRCs. Thus, access to biorepositories of human specimens should entail a contribution from researchers, either in the public or private sector, to the costs of collecting, annotating, storing, retrieving and processing of biospecimens. However, human biospecimens should not be sold in any circumstances. Regardless of the role of industry in core funding of BRCs, which is a matter of debate with serious implications, the responsibility for specimen collection and storage must remain within institutions. In defining mechanisms for BRC sustainability, there is a need to develop safeguards against exploitation and improper use of human biospecimens.

Recommendations for BRCs

Protection of persons

The first, basic requirement of a BRC is global safety. This includes protection of persons and of the environment against biological and chemical hazards, as well as protection of the data and information associated with the specimen collected. The management of these risks should be based on a general implementation of a precautionary principle similar to those used in laboratories and clinical settings, and should be embodied in a general safety management plan.

Biological hazards

All biological specimens should be considered as potentially infectious. Their collection and processing represents a source of hazard both for the person who is the source of the specimens and for the staff involved in these processes. Immunization of BRC staff is recommended when appropriate vaccines are available. In particular, immunization against the Hepatitis B Virus (HBV) is mandatory for staff involved in collecting and processing human blood or tissues. In addition, staff should be regularly checked on tuberculosis. Other significant risks are posed by exposure to the Hepatitis C Virus (HCV) and the Human Immunodeficiency Virus (HIV) as well as to the prion that causes Creutzfeld-Jacobs diseases. Further sources of biological risk have been identified (Grizzle and Fredenburgh, 2001).

General laboratory safety

In addition to biosafety, BRCs must follow strict general safety regulations and procedures in relation to chemical, physical and electrical safety. The use of liquid gases such as liquid nitrogen (LN2) for cryopreservation poses a serious source of hazard. Where LN2 refrigeration is employed, an adequate supply of refrigerant must be maintained. The supply maintained on site should be at least 20% more than the normal re-fill usage to allow for emergency situations.

Handling liquid nitrogen has serious safety implications. Sustained skin contact with LN2 can cause severe burns. In addition, nitrogen displaces oxygen, and the risk is inversely correlated to the size of the room. Oxygen level sensors should always be employed when LN2 freezers are used in a repository.

When bulk storage and piping systems are used, blockage of relief valves and/or overpressure may lead to simultaneous leakage of N2 from a number of relief valves, causing a “white-out” condition in a matter of a few seconds. This leads to a drop of visibility to almost zero and the oxygen level decreases in the area below what is necessary to sustain life. Personnel must evacuate immediately.

LN2 expands to 800 times its original volume at room temperature, causing a form of explosion hazard. Plastic and glass containers can easily explode if liquid is trapped when the container is removed from the LN2. Heavy gloves, a face shield and a protective garment should always be used under these conditions. Safety notices and protocols must be clearly displayed in the repository area. There are also risks associated with the use of chemical fixatives and solvents used in tissue processing. Electrical safety is an important concern. Deep-freezers must be properly wired to adequate sources of electrical supply, and grounded.

Work in a BRC also entails a number of occupational hazards typical of the laboratory environment. These risks must be taken into account before setting up a BRC, and their prevention must be integrated in all aspects of the standard operating procedures of the BRC.

Data management and informatics security

The protection of personal information and individual data associated with specimen collection is a fundamental requirement of a BRC. This should be achieved through the use of safe, structured bioinformatics systems. The mechanisms of access to these systems, as well as the permissions, should be clearly defined. Back-ups should be made on a regular basis to avoid data loss. The communication to third parties or authorities of data files containing personal information and identifiers should be strictly prohibited. Personal identifiers should be coded, and all individual data archived in the BRC management system should be protected with the same stringency as patient clinical files.

BRC documentation for scientific networking is also an important aspect. It is recommended that each BRC develop a website to inform the scientific community on the nature of its tissue banks and of its general content.

Biosecurity

The term “Biosecurity” refers to precautions that should be taken to prevent the use of pathogens or toxins for bioterrorism and biological warfare. Securing pathogens and toxins at research and diagnostic laboratories cannot prevent bioterrorism but can make it more difficult for potential terrorists to divert material from a legitimate facility so as to build a biological weapon. In 2006, WHO developed a document entitled “Biorisk management: Laboratory biosecurity guidance” (World Health Organization, 2006). The purpose of this document is to define the scope and applicability of “laboratory biosecurity” recommendations, narrowing them strictly to human, veterinary and agricultural laboratory experiments. The scope of laboratory biosecurity is broadened by addressing the safekeeping of all valuable biological materials, including not only pathogens and toxins, but also scientifically, historically and economically important biological materials such as collections and reference strains, pathogens and toxins, vaccines and other pharmaceutical products, food products, genetically modified organisms (GMOs), non-pathogenic microorganisms, extraterrestrial samples, cellular components and genetic elements.

Laboratory biosecurity measures should be based on a comprehensive programme of accountability for valuable biological material that includes : regularly updated inventories with storage locations, identification and selection of personnel with access, plan of use of valuable biological material, clearance and approval processes, documentation of internal and external transfers within and between facilities and on any inactivation and/or disposal of the material. Likewise, institutional laboratory biosecurity protocols should include how to handle breaches in laboratory biosecurity, including: incident notification, reporting protocols, investigation reports, recommendations and remedies. Adoption of these security requirements is important for BRCs maintaining pathogenic or toxic biospecimens.

General considerations for establishing a BRC

A number of factors must be taken into account when setting up and running a BRC. A detailed description of these requirements can be found in the “Best Practices for Biological Repositories” developed by the International Society of Biological and Environmental Repositories” (International Society for Biological and Environmental Repositories, 2005). The paragraph below underlines aspects of particular importance in setting up a BRC for cancer research.

Institutional commitment

Many factors contribute to the decision to develop and run a BRC. In practice, the process often starts from the willingness of medical doctors and scientists to develop a resource useful for diagnosis, prognosis and research purposes. However, initiating a BRC must not only rely on individual action but also requires a clear commitment by the institution. It also needs to ensure that collections are developed within appropriate legal, ethical, clinical, scientific and technical guidelines to provide historical continuity in specimen and record keeping. Finally, the BRC should ensure that the materials stored by the BRC can be made available for research.

The purpose of the BRC must be clearly formulated and documented. BRCs that contract with third parties for laboratory service should keep detailed records of the nature of the contract, of the identity of the contractor and of the inclusive dates of the contract period. In case of loss of funding or

other adverse events that may prevent the institution maintaining its commitment, it is the responsibility of the institution to take the necessary steps to transfer collected specimens and data to another institution that will take over the commitment to long-term maintenance of the collection.

BRC management and staff

BRCs should be adequately staffed, and the personnel selected for these tasks must have an appropriate level of specialized training. The BRC should be placed under the overall supervision of a biological resource manager with sufficient training, experience and seniority to fulfil the scope of the activities of the BRC. The manager is responsible for operations, including compliance with current regulations. The manager has a critical role in receiving, processing and answering requests for access to stored specimens.

Running a BRC requires dedicated staff for specimen processing and storage and for data management. The job description, tasks and reporting system of all supervisory and technical staff involved in the BRC must be documented. This is of particular importance in instances where the staff involved in the BRC also performs other tasks within the institution (e.g. pathology service or service activities in molecular biology). Staff must have adequate educational background, experience and training to ensure that assigned tasks are performed in accordance with the BRC's established procedures.

Infrastructure and facilities

The BRC infrastructure depends upon the types of material being stored, the required storage conditions, the projected retention periods and the projected use of the materials.

1. BRCs should have dedicated facilities that are not shared with other activities. Sufficient air conditioning must be provided for air circulation and to maintain ambient temperature ≤22°C at the level of the freezers/refrigerators in order to prevent excess freezer wear and early failure. Rooms that contain LN2 tanks should be equipped with appropriate air flow systems coupled to an oxygen level alarm system to avoid the accumulation of N2 in case of leakage (see below). Storage facilities and instruments should be monitored and supported by appropriate alarm systems *(Figures 1 and 2).*

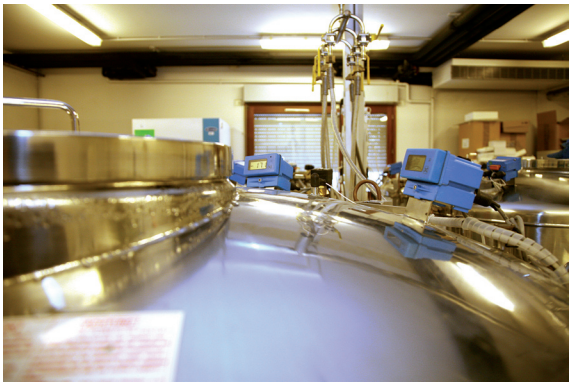

Figure 1

Figure 2

2. BRCs should be equipped with a system that adequately limits access to appropriate staff and protects against physical intrusion. In principle, only persons assigned to the BRC operation should have access to the material, and all materials added to or withdrawn should be documented *(Figures 3 and 4).*

Figure 3

Figure 4

3. BRCs require a constant source of electrical power. Given that all commercial power will fail at some time, a backup power system is required. The most common type of backup power is the motor generator. Such a system should have the capacity to run for sufficient time to allow the restoration of power supply (typically 48–72 hours) and should be regularly tested.

4. Adequate backup capacity for low-temperature units must be maintained. The total amount of backup storage required for large repositories must be determined empirically, but will typically be 5%– 10% of the total freezer capacity.

5. Every repository should employ basic security systems. The systems must be monitored and alarms responded to 24 hours per day, 7 days per week. Response systems must be in place to ensure that a responsible individual can take the necessary action to respond to an alarm in a time frame that prevents or minimizes loss or damage to the collection materials. Systems should allow for calls to other key staff from a list of staff phone numbers if the first individual fails to acknowledge the alarm.

6. Whenever possible, it is recommended to consider splitting stored biospecimens into two sets of aliquots, each set stored in a different location. This strategy will avoid unnecessary loss in case of adverse events in one location. For multicentre studies it is recommended that each collection centre retain a set of aliquots at the place of collection, with the other set transported to another location which is common for all participant centres.

Storage conditions

Biospecimens should be stored in a stabilized state. In selecting the biospecimens' storage temperature, consider the types of biospecimens, the anticipated length of storage, the biomolecules of interest and whether goals include preserving viable cells. Some other conditions should be considered such as humidity level, light, etc.

Cryopreservation

Cryopreservation is the recommended standard for preservation of human biological samples for a wide range of research applications. Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures, typically -80°C (freezer) or -196°C (nitrogen liquid phase) *(Figure 5).*

Figure 5

At these low temperatures, any biological activity, including the biochemical reactions that would lead to cell death, is effectively stopped. However, due to the particular physical properties of water, cryopreservation may damage cells and tissue by thermal stress, dehydration and increase in salt concentration, and formation of water crystals. Table 2 lists the most commonly accepted cryopreservation standards for human tissue and body fluids. Specific applications (e.g. proteomics or storage of primary cell cultures) may require more complex cryopreservation procedures. General information on the principles of cryopreservation may be found at http://www.cryobiosystem-imv.com/CBS/Cryobiology/cons_cbs.asp (CryoBioSystem, 2002).

Specimen freezing is generally performed by placing the specimen in a sealed (but not airtight) container and immersing the container in the freezing medium. The ideal medium for rapid freezing is isopentane that has been cooled to its freezing point (-160°C). To achieve this, the vessel containing the isopentane should be placed in a container of liquid nitrogen. The freezing point approximately corresponds to the moment when opaque drops begin to appear in the isopentane. Direct contact of the specimen with liquid nitrogen should be avoided, as this can damage tissue structure.

Figure 6

Temperature in °C	Properties of water/ liquid nitrogen	Cryopreservation method	Biological relevance
0 to +4	Ice melting	Refrigerator	Processing of fresh material
-0.5 to -27	Ice fusion area	Freezer	
-27 to -40	Ice	Freezer	Limit of protein mobility/ DNA stability
-40 to -80	Limit of water molecules mobility	Freezer	RNA stability
-80 to -130	Ice transition	Freezer/liquid nitrogen	Recommended storage for blood and urine
-130 to -150	Liquid nitrogen (vapour phase)	Liquid nitrogen	Recommended storage of tissues
-150 to -196	Liquid nitrogen (liquid phase)	Liquid nitrogen	Possible micro-fractures Recommended storage of living cells

Table 2 : Basic standards of cryopreservation and applications to biological specimens

Other fixation and preservation methods

Formalin or alcohol fixation and paraffin embedding may be used as an alternative method to preserve tissues at relatively low cost when adequate freezing procedures and storage facilities are not available. Fixed paraffin blocks may be stored in light- and humidity-controlled facilities at 22°C *(Figures 6 and 7).*

Tissues fixed according to strict protocols may be used for DNA extraction. The DNA is usually fragmented but remains suitable for PCR-based analysis of short DNA fragments (up to 1–2 kbp). However, fixed tissues are of limited usefulness for RNA extraction.

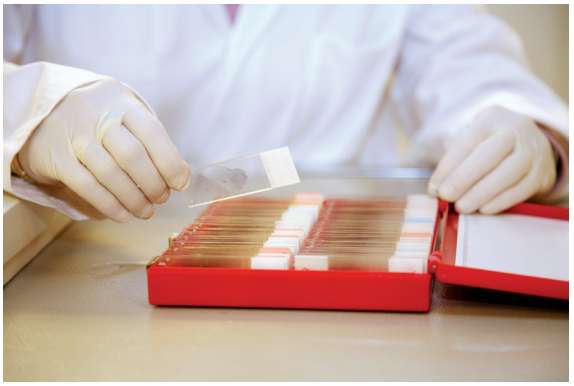

Figure 7

RNAlater is a commercial aqueous, non-toxic tissue storage reagent that rapidly permeates tissues to stabilize and protect cellular RNA. RNAlater eliminates the need to immediately process tissue samples or to freeze samples in liquid nitrogen for later processing. Tissue pieces can be harvested and submerged in RNAlater for storage for specific periods without jeopardizing the quality or quantity of RNA obtained after subsequent RNA isolation. However, specimens once placed in RNAlater cannot be further used for histomorphopathological analyses.

Liquid nitrogen (LN2) tanks

The critical temperature for sensitive tissues, organisms and cells is generally considered to be -132°C, the glass transition temperature (Tg) (Eiseman et al., 2003). Vapour-phase storage is preferred over liquid-phase storage, but the design of the tank/ freezer is critical to maintain a sufficient amount of LN2 in the vapour phase. Use of vapour phase avoids some of the safety hazards inherent in liquid-phase storage, including the risk of transmission of infectious agents. In contrast, liquid-phase storage necessitates less frequent resupply of LN2 and thus affords better security in case of a crisis in LN2 supply *(Figures 8 and 9).*

Figure 8

Mechanical freezers

Mechanical freezers are employed in a variety of storage temperature ranges, including -20°C, -40°C, -70°C to -80°C, and -140°C. Freezers should be equipped with alarms set at about 20°C warmer than the nominal operating temperature of the unit. Adequate back-up capacity at standby is needed *(Figures 10 and 11).*

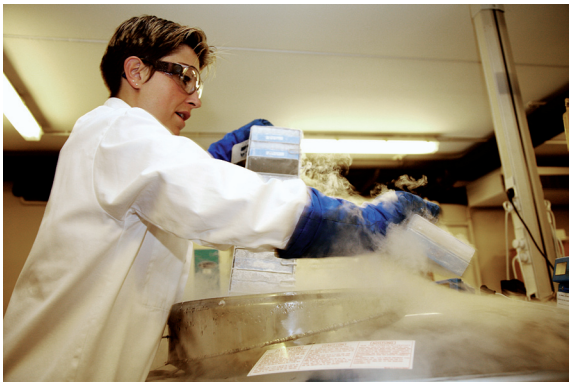

Figure 9

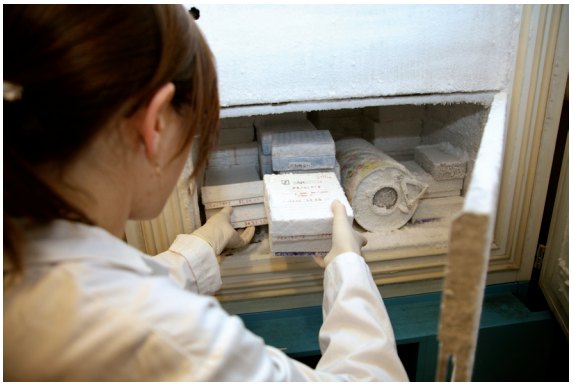

Figure 10

Figure 11

Dry ice

Dry ice or solid-phase carbon dioxide is frequently used as a refrigerant for shipping and

emergency backup for mechanical freezers. Handling precautions should be employed when handling this material, which exists at a nominal -70°C. As dry ice sublimates, the CO2 level in the surroundings can increase. In confined areas the carbon dioxide can displace oxygen, presenting an asphyxiation hazard.

Standard operating procedures

BRCs should develop, document and regularly update policies and procedures in a standardized written format incorporated into a Standard Operating Procedures (SOP) Manual that is readily available to all laboratory personnel. The SOP Manual should specifically include:

- Specimen handling policies and procedures including supplies, methods and equipment;
- Laboratory procedures for specimen processing e.g. aliquoting, tests; quality control;
- Policies and procedures for shipping and receiving specimens;
- Records management policies;
- Quality assurance (QA) and quality control (QC) policies and procedures for supplies, equipment, instruments, reagents, labels, and processes employed in sample retrieval and processing;
- Emergency and safety policies and procedures, including reporting of staff injuries and exposure to potential pathogens;
- Policies and procedures for the investigation, documentation and reporting of accidents, errors, complaints and adverse outcomes;
- Policies and procedures and schedules for equipment inspection, maintenance, repair and calibration;
- Procedures for disposal of medical and other hazardous waste; and
- Policies and procedures describing requirements of training programs for BRC staff.

BRCs should have appropriate quality assurance (QA) and quality control (QC) programmes regarding equipment maintenance and repair, staff training, data management and record keeping, and adherence to Good Laboratory Practice. All BRC operations must be subject to regular audits. The timing, scope and outcome of these audits should be documented. QA is an integrated system of management activities involving planning, implementation, documentation, assessment and improvement to ensure that a process or item is of the type and quality needed for the project. QC is the system of technical activities that measures the attributes and performances of a process or item against defined standards, to verify that the stated requirements are fully met.

Records Management

The importance of adequate data management cannot be overstated. There is an unmet need for the development of open-source software for BRC management. BRCs must develop a record management system that permits detailed records to be made concurrently with the performance of each step in the collection, processing and distribution of specimens. This may include but is not limited to : informed individual consent, procurement, processing, preservation, quarantining, testing, record review, releasing, labelling, storage, distribution, and quality control of specimens. Records should be created and maintained in a manner that allows full traceability. Laboratory Information Management System (LIMS) allows the management of such data. Data security systems should be adequate to ensure confidentiality and safety. Record management should be regularly audited. Records should be kept for at least 10 years after expiration of specimen storage or specimen distribution. Electronic records should be adequately protected (regular backups on appropriate media, intrusion-proof management systems).

The BRC inventory should be checked as part of quality assurance and quality control programmes at regular intervals (e.g. every two years) to assess the concordance between stored specimens and records. The specific position of every stored aliquot should be tracked. Each freezer, refrigerator or room temperature storage cabinet should have a unique identifier. A convention should be established for numbering shelves, racks and boxes as well as each location within the container. The biorepository database should be updated each time a biospecimen is moved within or out of the biorepository.

Specimen labelling

Each specimen should be labelled in such a manner that the labelling will survive all potential storage conditions, in particular dry ice and liquid nitrogen.

1. Ink used on the label should be resistant to all common laboratory solvents. It is recommended to print labels with a barcode (linear or 2D), thus providing a direct link to database software. However, it is also essential to include human-readable indications of contents. The barcode template should be documented. The software used for labelling should allow data import and export in standard formats *(Figure 12).*

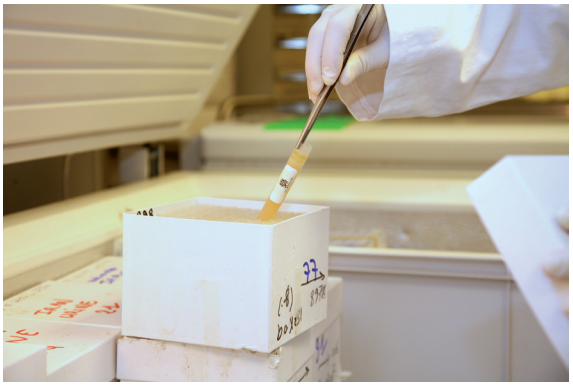

Figure 12

2. All specimens should ideally be labelled with at least two human-readable forms of identification without revealing the identity of the donor.

3. Information on the label should include the tissue bank's unique identifier number and/or the number of the place within the storage system, with the same information repeated in the barcode if available.

Specimen collection, processing and storage

Many types of biological material can be stored for cancer research purposes. The methods used to collect biospecimens will vary depending on what the intended end use is and how the specimens will be processed. This paragraph provides general advice for collection of whole blood, blood cells, plasma/serum, solid tissues, urine, buccal cells and saliva. These recommendations are derived from those presented in the Biorepository Protocols of the Australasian Biospecimen Network (Australian Biospecimen Network, 2006). http://www.abrn.net/pdf/ABN_SOPs_Review_Mar06_final.pdf.

Collection of blood or blood-derived products

Blood

Detailed instructions and protocols for collection of blood or blood derivatives are given in the Selected Protocols section. The following general guidelines should be considered.

1. All blood should be treated as potentially infectious. Blood samples for research purposes should be collected concurrently with taking of routine clinical blood samples, so as to limit discomfort to individuals. Blood should be collected from fasting individuals after 8–12 hours of absence of food, alcohol and caffeine-containing beverages.
2. Blood should not be collected after prolonged venous occlusion.
3. Tubes into which the blood is collected should be clearly labelled. *(Figure 13)*

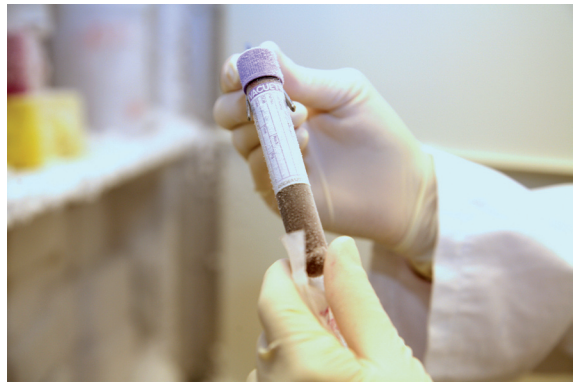

Figure 13

4. For the preparation of plasma, blood may be collected into EDTA, ACD (Acid Citrate Dextrose), lithium heparin, or into a clotting tube containing separating gel.
5. Ideally, blood should be processed within 1 hour of collection. After that time, cell viability decreases rapidly, resulting in poor cell structure and degradation of proteins and nucleic acids.
6. If a long time elapses between collection and processing (2–3 hours) it is recommended to use ACD tubes.
7. Lithium heparin is generally used if cytology studies have to be performed, but it is not recommended for proteomics work.
8. PCR was clearly interfered with when

heparinised blood was used as a source of template DNA (Yokota et al., 1999)

9. Either EDTA or ACD tubes can be used if DNA is to be extracted or lymphoblastoïd cell lines to be derived. Lithium heparin is not recommended for proteomics application and lymphoblastoïd cell lines establishment.

10. EDTA tubes are recommended if protein studies will be performed. The use of EDTA tubes results in less proteolytic cleavage than heparin and citrate-anticoagulated plasma.

11. For the preparation of plasma, the blood should be centrifuged as soon as possible. For the preparation of serum, the blood should be processed within 1 hour after collection.

12. The amount of blood collected should be justified when applying for ethical clearance.

13. Reduced volume of blood in a tube containing additives should be recorded to avoid confounding results.

14. The time and date of blood collection and time of freezing should be recorded, as well as any deviations to the standard processing protocol.

15. Blood should be transported at room temperature or on melting ice depending on the particular applications. Samples to be used for proteomics assays should be processed immediately at room temperature. Cool temperature can activate platelets and release peptides into the samples ex-vivo.

16. Blood spot collection should be considered an alternative to whole blood when conditions necessitate easier collection and cheap room-temperature storage (Yokota et al., 1999;Steinberg et al., 2002). Different types of collection cards are available (Guthrie cards, FTA cards, Isocode) (see Selected Protocols section).

"Guthrie cards" (903 filter paper, Schleicher and Schuell) are used to collect heelstick blood from newborns for metabolic disease screening. The 903 paper is manufactured from 100% pure cotton linters with no wet-strength additives. The critical parameters for newborn screening sample collection are blood absorbency, serum uptake and circle size for a specified volume of blood. Blood spots archived as long as 17 years, sometimes at room temperature, have also provided valuable sources of amplifiable DNA (Makowski et al., 1996) *(Figure 14).*

Figure 14

Modified cards (Isocode® or FTA cards®) have been developed consisting of filter paper impregnated with a proprietary mix of chemicals that serves to lyse cells, to denature proteins, to prevent growth of bacteria and other microorganisms, and to protect nucleic acids from nucleases, oxidation and UV damage. Room temperature transport in folders or envelopes (by hand or mail) has been common for years. The papers protect DNA within the samples for some years at ambient conditions. The main variable is expected to be the quality of the storage atmosphere, particularly the content of acid gases and free-radical-generating pollutants, although FTA® paper can protect against such conditions (Smith and Burgoyne, 2004). Genomic DNA stored on FTA® Cards at room temperature for over 14 years has been successfully amplified by PCR. In contrast, genomic DNA stored at room temperature on non-FTA Cards for over six months did not amplify. Sample integrity is optimized when FTA cards are stored in a multi-barrier pouch with a desiccant packet (Mbogori et al., 2006).

Buffy Coat

For DNA testing, if DNA cannot be extracted from blood within three days of collection, the buffy coat may be isolated and stored at -70°C or lower prior to DNA isolation. Buffy coat specimens that are being used for immortalization by Epstein-Barr virus should be transported frozen on dry-ice. RNA should be isolated from buffy coat within one to four hours of specimen collection; alternatively RNA stabilization solution (e.g. RNAlater) should be used (see Selected Protocols section).

Collection of solid tissues

Solid tissues are collected by biopsy or during surgical procedures. Collection should be carefully planned with surgeons, clinical staff and pathologists. All materials and instruments should be prepared in advance. Detailed guidelines are presented in the Selected Protocols section.

1. The collection of samples for research should never compromise the diagnostic integrity of a specimen. Only tissue which is excess to diagnostic purposes can be collected for the resource.
2. All tissue should be treated as potentially infectious; the collection process should be carried out in the most aseptic conditions possible.
3. The intact surgical specimen or biopsy should be sent to pathology as soon as possible.
4. It is recommended that surgical specimens or biopsy be preserved within 1 hour of excision; however, tissue subject to a delay up to 2 hours should still be collected (Eiseman et al., 2003). Detailed record of the timing of events from excision to fixation or freezing should be kept.
5. Each specimen receptacle must be clearly labelled. *(Figure 15)*

Figure 15

6. Transfer of specimens must be carried out as soon as possible in order to minimize the effect of hypoxia upon gene expression, degradation of RNA, proteins and other tissue components.
7. For transport from surgery to pathology, or to the repository, fresh specimens should be placed in a closed, sterile container on wet ice.
8. A pathologist should supervise the procurement of the tissue. The pathologist will examine the sample, and, allowing adequate tissue for diagnosis, will remove a portion of the tumour and adjacent normal tissue. If applicable, involved lymph nodes and metastasis will also be collected. Tissues must be sliced with sterile forceps and scalpel blades, and the staff must use sterile gloves.
9. Tissue bank staff must be present in pathology to freeze or fix the tissue as quickly as possible. Tissues must be snap frozen either directly or enclosed in a container immersed in the freezing medium (e.g. precooled isopentane). Liquid nitrogen is not recommended as a suitable freezing medium for direct snap freezing due to the potential formation of cryo-artefacts.
10. When dry ice/liquid nitrogen is not readily available, tissue collections into RNAlater may be a good alternative provided that this tissue is not required for diagnostic purpose, and permission is given by the pathologist.

Collection of other specimens

Urine

Urine is easy to collect and is a suitable source of proteins, DNA and other metabolites. Urine should be routinely stored at -80°C. Ambient temperature storage before freezing should be kept to a minimum (see Selected Protocols section).

Buccal cells

The collection of buccal cells is not difficult and does not require highly trained staff. Buccal cell collection is considered when non-invasive, self-administered, or mailed collection protocols are required for DNA analysis (Steinberg et al., 2002). However, buccal cell collection will yield only limited amounts of DNA in comparison to blood.

Different methods of self-collection are available depending on the endpoints and the analyses to be performed. (Mulot et al., 2005).

Cytobrush

This method is to collect cells on a sterile cytobrush by twirling it on the inner cheek for 15 seconds. The operation is repeated three times, on the two cheeks. The swabs are separated from the stick with scissors and transferred to a cryotube. The duration of the collection can influence the DNA

yield. Garcia-Closas et al. reported that cytobrushes produce DNA with good quality (Garcia-Closas et al., 2001). However King et al. concluded that mouthwash is superior for reactions requiring long fragments (King et al., 2002).

Mouthwash

Buccal cells are collected by rinsing the mouth for 10 seconds with 10ml of sterile water and expectorating the rinse into a 50ml centrifuge tube. This operation is repeated three times. The amounts of extracted DNA can vary according the time of brushing. The effect of lag time of storage at room temperature is visible for mouthwashes, while the cytobrushes are less sensitive to the lag time at room temperature (see Selected Protocols section).

Treated cards

These cards are treated to inhibit the growth of bacteria and kill viruses thereby minimizing nucleic acid degradation. The individuals expectorate saliva into a sterile cup. The tip of the treated card triangle is placed into the saliva, which is wicked onto the matrix. The treated card is air-dried and placed in a bag with dessicant. Treated cards correspond to the lowest efficiency for DNA yield because of the small quantity of collected saliva. Moreover, some proteins are let in the solution of extracted DNA. Therefore the DNA can not be kept for long-term conservation. An advantage for this method of buccal cells collection is its low cost due to the absence of an extraction step. Finally, cytobrushes and mouthwashes are generally considered unsuitable for children because cytobrushes are abrasive. Mouthwashes require participants to expectorate and may be aspirated or swallowed.

Saliva

Saliva is used as a biological fluid for the detection of different biomarkers such as proteins, drugs and antibodies. Saliva meets the demand for non-invasive, accessible, and highly efficient diagnostic medium. The sample collection is non-invasive (and thus not painful) and can easily be done without various devices. Whole saliva is collected by expectoration into a provided tube, while for the collection of submandibular saliva and sublingual saliva different ducts need to be blocked by cotton gauze (See figures 16 and 17 below). For the collection of paratoid saliva, a paratoid cup should be used (World Health Organization, 2007b) (see Selected Protocols section).

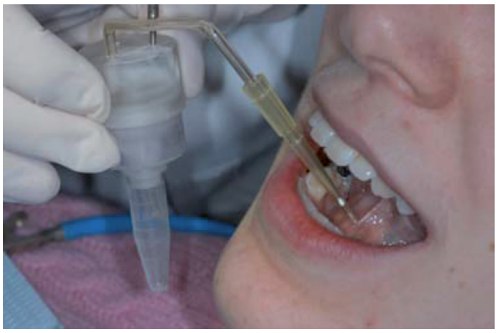

Figure 16

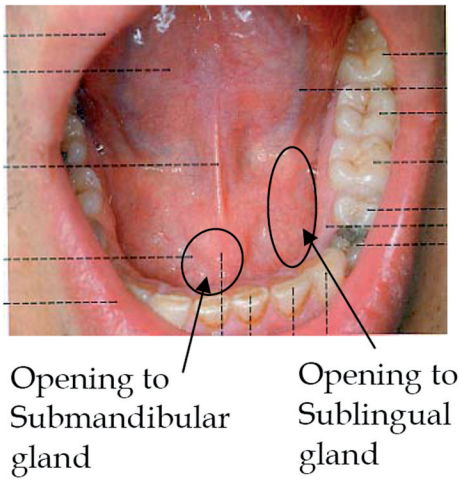

Figure 17

Bronchoalveolar lavage (BAL)

The airways, and particularly the alveoli, are covered with a thin layer of epithelial lining fluid (ELF), which is a rich source of many different cells and soluble components of the lung that play important functions by protecting the lung from undue aggressions and preserving its gas-exchange capacity (Robbins and Rennard, 1996). Bronchoalveolar lavage performed during fiber-optic bronchoscopy is the most common way to get samples of ELF (Reynolds, 2000). The cellular and protein composition of the ELF reflects the effects of the external factors that contact the lung and is of primary importance in the early diagnosis, assessment and characterization of lung disorders as well as in the search for disease markers (Griese, 1999).

Bronchoalveolar lavage is classically performed by instillation of buffered saline solution divided into 3–4 aliquots (typically a total volume of 100–150ml) through a flexible fiberoptic bronchoscope, after local anesthesia. The first 10ml should be processed separately and is denoted as bronchial lavage (BL).

The rest of the lavage, denoted as bronchoalveolar lavage (BAL), should be pooled into a sterile siliconised bottle and transported on ice immediately to the laboratory. At the laboratory, the total volume of the lavage is measured, and cells and proteins are separated by centrifugation. The lavage fluid should be frozen and stored at -80°C until use.

Bone Marrow Aspirate (BMA) and Fine Needle Aspirate (FNA)

The Regional Office for South-East Asia at the World Health Organization has published several publications on Blood Safety and Clinical Technology. Apart from the Australian Biospecimen Network recommendations, the following paragraphs on bone marrow aspirate and cerebrospinal fluid are derived from the publications "Guidelines on Standard Operating Procedures for MICROBIOLOGY" and "Guidelines on Standard Operating Procedures for CLINICAL CHEMISTRY" (World Health Organization, 2007a; World Health Organization, 2007b).

Bone marrow is the soft tissue found in the hollow interior of bones. In adults, marrow in large bones produces new blood cells. There are two types of bone marrow: red marrow (also known as myeloid tissue) and yellow marrow. In cancer research red bone marrow from the crest of the ilium is typically examined.

Bone marrow should be collected by a doctor who is well trained in this procedure. Bone marrow should be aspirated by sterile percutaneous aspiration into a syringe containing an EDTA anticoagulant, and the specimens should be chilled immediately. Heparin is not recommended as an anticoagulant for molecular testing. If a specimen contains erythrocytes, it should be processed to remove the erythrocytes before freezing. The bone marrow samples should be freshly frozen and stored at -80°C.

Cerebrospinal fluid (CSF)

Cerebrospinal fluid (CSF) originates from the blood. The choroid plexes in the 1st, 2nd and 3rd ventricles of the brain are the sites of CSF production. CSF is formed from plasma by the filtering and secretory activities of the choroid plexus and lateral ventricles. CSF circulates around the brain and the spinal cord. CSF nourishes the tissues of the central nervous system and helps to protect the brain and the spinal cord from injury. It primarily acts as a water shock absorber. It totally surrounds the brain and the spinal cord and thus absorbs any blow to the brain. CSF also acts as a carrier of nutrients and waste products between the blood and the central nervous system (CNS).

CSF is the most precious biological material. Often, only small volumes of CSF are available for analysis due to difficulty in collection. Hence handle this with care. Only a physician or a specially trained nurse must collect the specimen. After sampling the specimen should be transferred into a clean penicillin vial containing about 8mg of a mixture of EDTA and sodium fluoride in the ratio of 1:2. Centrifuging of CSF is recommended before freezing if the sample contains red blood cells or particulate matter. The specimen should be frozen and stored at -80°C or in liquid nitrogen. Do not delay freezing the CSF because cells are rapidly lysed once the CSF is removed from the body.

Semen

Seminal fluid is the liquid component of sperm, providing a safe surrounding for spermatozoa. At pH 7.35-7.50, it has buffering properties, protecting spermatozoa from the acidic environment of the vagina. It contains a high concentration of fructose, which is a major nutriment for spermatozoa during their journey in the female reproductive tract. The complex content of seminal plasma is designed to ensure the successful fertilization of the oocyte by one of the spermatozoa present in the ejaculum. Seminal plasma is a mixture of secretions from several male accessory glands, including prostate, seminal vesicles, epididymis, and Cowper's gland (Pilch and Mann, 2006).

After collecting ejaculate, the fresh ejaculate should immediately be spun down at 4°C to separate seminal fluid from spermatozoa. Protease inhibitors should then be added to the sample to avoid digestion by powerful proteases present in seminal fluid. To ensure complete separation of cell debris or occasional spermatozoa from seminal plasma, the sample can be centrifuged a second time. The

sample should be stored at -80°C.

Cervical and urethral swabs

The quality of collected cervical and urethral specimens depends on appropriate collection methods. Swabs, brushes or other collection devices should be placed in a transport medium, or transported dry in a sealed tube and resuspended in the transport medium upon arrival. The transport fluid may either be stored at -70°C or lower or immediately centrifuged, and the pellet processed for DNA or RNA extraction (see Selected Protocols section).

Hair

Currently, hair analysis is used for purposes of assessing environmental exposures. Hair analysis is also used to test for illegal drug use and to conduct criminal investigations (see Selected Protocols section).

Nail

Nail clippings may contain analytes of interest that were deposited during the growth of the nail. Nail specimens can be collected for drugs, nutritional, poisons and toxicity testing (see Selected Protocols section).

Specimen annotations, data collection

It is recommended that BRCs adopt standardized systems for annotating the characteristics of collected specimens as well as data on the patients or individuals who are the source of these specimens. The nature and extent of data collection may vary depending upon the nature and purpose of the research, the type of cancer and nature of specimen collected. The paragraphs below provide a brief outline of the structure of minimal annotation datasets.

Annotations on patients/individuals

It is recommended to obtain the following information from individuals for a better characterization of them.

1. Local coded personal identifier
2. Disease status (normal, cancerous)
3. Tumour topography according to the International Classification of Disease – Oncology (ICD-O 3rd edition) *(Figure 18)*

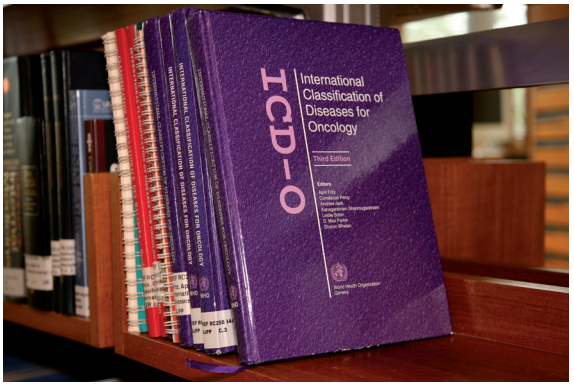

Figure 18

4. Tumour morphology according to ICD-O 3rd edition
5. TNM staging (if applicable)
6. Tumour grade (if applicable)
7. Diagnostic date (if applicable)
8. Diagnostic description (if applicable)
9. Age at time of specimen collection (in years)
10. Gender
11. Place of residence (city/region/country)
12. Ethnicity/language spoken or place of origin/ birth of parents/grandparents
13. Information on exposure and risk factors (if applicable)
14. History of cancer disease (if applicable)
15. Evidence for familial history of cancer (if applicable)
16. Involvement in clinical trial/cohort study
17. If appropriate, information on medical history, treatment with past and current medication and response to therapy, concomitant disease, secondary tumours/Laboratory data
18. If appropriate, information on perioperative medication and treatment should be included as optional information:
    - Protocol name
    - Prior treatment
    - Treatment type (surgery, chemotherapy, radiotherapy….)
    - Treatment start date
    - Treatment end date
    - Treatment response date
    - Treatment response type (none, low, average, good, complete….)

19. If appropriate, information on duration of follow-up and disease outcome:
    - Relapse date
    - Relapse type (localized, distant…)
    - Death date
    - Duration of global survival
    - Duration of survival without relapse
20. Reference to the informed consent and its scope
21. Hazard status

Whenever coded links with the patients clinical files are maintained, the annotations can take the form of "yes" or "no" answers in relation to the informed consent in the clinical file.

Annotations on stored specimen

The following criteria should be recorded for better characterization of biospecimens.

1. Storage centre identification
2. Local BRC inventory code
3. Nature of specimen
4. For solid tissue, tissue condition (tumour/non-tumour/interface)
5. Packaging
6. Number of aliquots/quantity of biological material
7. Preservation protocol
8. Time elapsed between tissue removal and preservation (if applicable)
9. Date of specimen collection/storage
10. Record of storage incidents
11. Temperature during transport
12. Storage temperature
13. Storage conditions (agents added to the sample)
14. Documentation on processing method
15. History of freezing/thawing
16. Amount of tissue collected, and amount left over in storage

Quality control

Quality check protocols must be incorporated as part of each Standard Operating Procedure (SOP) carried out by the BRC. In addition, it is necessary to adopt quality control procedures to address the quality of the BRC on a general basis. The following aspects should be taken into account:

1. Staff training: the competency of staff to perform tasks according to SOPs should be checked on a regular basis (e.g. annually).
2. Infrastructure and equipment maintenance: a preventive maintenance plan should be adopted. Equipment usage should be monitored through logbooks reporting daily operations and incidents.
3. Safety and contingency plans: alarm systems and alarm response procedures should be tested on a regular basis; detailed debriefing should be held after any incident to identify possible preventive actions and to improve emergency responses. In large facilities, it is recommended to run safety exercises.
4. Assessment of specimen quality, electronic records and storage location: it is recommended that up to 1% of the specimen content of the biobank be checked annually. This check should include (1) the physical verification of the specimen location and of the durability of the storage vessels, (2) the verification of annotations and data records, and (3) in the case of collections that are not being actively exploited, the verification of the biological quality of the specimens (extraction and analysis of DNA, RNA and other biomolecules as appropriate).

Quality control results must be recorded and made available for examination upon request by internal or external auditors.

Specimen shipping

Human biospecimens are considered as "dangerous goods", that is, "articles or substances which are capable of posing a risk to health, safety, property of the environment". According to UN regulations, dangerous goods meet the criteria of one or more of nine UN hazard classes (see links to references below). The relevant class for biological specimens is Class 6, division 6.2: Infectious substances.

The shipping and dispatch of biospecimens is subject to international regulations. These regulations, applicable to any mode of transport are based upon the Recommendations by the Committee of Experts on the Transport of Dangerous Goods (UNCETDG),

a committee of the United Nations Economic and Social Council.

The *Technical Instructions for the Safe Transport of Dangerous Goods by Air* published by the International Civil Aviation Organization (ICAO) are the legally binding international regulations. The International Air Transport Association (IATA) publishes Dangerous Goods Regulations (DGR) that incorporate the ICAO provisions and may add further restrictions (International Air Transport Association, 2003). The ICAO rules apply on all international flights. For national flights, i.e. flights within one country, national civil aviation authorities apply national legislation. This is normally based on the ICAO provisions, but may incorporate variations. State and operator variations are published in the ICAO Technical Instructions and in the IATA Dangerous Goods Regulations *(Figure 19).*

Figure 19

The following links refer to these regulations :
UNECE (United Nations Economic Commission for Europe)
UN Recommendations on the Transport of Dangerous Goods. Model Regulations.
http://www.unece.org/trans/danger/publi/unrec/rev13/13files_e.html

IATA (International Air Transport Association)
Dangerous Goods Regulations 2005.
http://www.iata.org/ps/publications/9065.htm

ICAO (International Civil Aviation Organization)
http://www.icao.int/icao/en/m_publications.html

WHO (World Health Organization)
Transport of infectious substances 2005
http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2005_22r%20.pdf

Each person involved in the transport of biospecimens classified as dangerous goods by IATA, should follow and validate a training session. It concerns persons involved in the preparation of documentation but also persons involved in packaging biospecimens.

When preparing to transport biospecimens, it is important to consider shipping time, distance, climate, season, method of transport, and regulations as well as the type and number of biospecimens to be sent and their intended use. Some general guidelines and regulations are included below.

Regulations

Infectious substances fall into two categories. *Category A* comprises substances which are transported in a form that, when exposure to them occur, are capable of posing permanent disability, life-threatening, or fatal disease to humans or animals. Category A specimens include, but are not restricted to, specimens contaminated by highly pathogenic viruses (Ebola, Hantaan, Marburg, Lassa, etc.) or cultures of viruses such as Dengue, Human Immunodeficiency Virus (HIV) or Hepatitis B Virus (HBV). The proper shipping name for such substances is UN2814: “Infectious substances affecting humans” or UN2900: “Infectious substances affecting animals only”. *Category B* comprises substances that do not meet the above criteria. Most human specimens such as blood samples, tissues, saliva, exfoliated cells or urine not contaminated by highly pathogenic viruses will fall into Category B. The proper shipping name for such substances is UN3373: “Biological Substance, Category B” (note: before the 1st of January 2007 these shipping names were “Diagnostic Specimens” or “Clinical Specimens”).

Biospecimens or derived products that have been specifically treated to neutralize infectious agents, or for which there is a minimal likelihood that pathogens are present, are not subject to these regulations. The proper shipping name for such substances is “Exempt Human (or Animal) Specimens”.

Packaging

The basic triple packaging system applies to all substances. It consists of three layers as follows:

- *Primary receptacle:* a primary watertight, leak-proof receptacle containing the specimen, packaged with enough absorbent material to absorb all fluids in case of breakage;
- *Secondary packaging:* a second, durable watertight, leak-proof packaging to enclose and

protect the primary receptacle. Several primary receptacles may be placed in one secondary packaging but sufficient additional absorbent material should be use to absorb all fluid in case of breakage;

- *Outer packaging:* an outer, shipping packaging of suitable, cushioning material, protecting its contents from outside influences while in transit.

Figure 20

Figure 21

(Figures 20 and 21)

Use appropriate insulation; e.g. for 8°C to -20°C use gel packs, for -80°C use dry ice, and if samples need to be kept at -150°C, transport them in a dry shipper containing liquid nitrogen. Ensure enough refrigerant is included to allow for a 24-hour delay in shipping.

The triple packaging system also applies to Exempt Human Specimens such as Guthrie cards (that should be transported in watertight plastic bags) or histopathological slides (that need to be cushioned to prevent breakage). In all cases, desiccants should be used for samples sensitive to humidity.

Labelling

All outer packages must bear a United Nations packaging specification marking according to the category in which the specimens fall. For category A, the packaging instruction Pi602 applies. For category B, the relevant packing instruction is Pi650. Detailed instructions are described in the IATA «Dangerous Goods Regulations, 2007 (International Air Transport Association, 2007)».

When shipping biospecimens overseas, the sender must be aware of the requirements and regulations in the destination country prior to the initiation of the shipment, and ensure that the consignment adheres to these regulations.

Access to stored materials and data for research purposes

Access to human biological specimens for research purposes is crucial for most fields of cancer research and in particular to genomics, proteomics, metabolomics or molecular imaging. Each BRC should establish clear guidelines for distribution and sharing of biospecimens and data, compatible with local, national and international prevailing laws, ethical principles and protection of intellectual property rights. However, BRCs should not serve exclusively to satisfy individual needs or research projects and all efforts should be made to ensure specimens and data are available to the wider scientific community. So far little has been done internationally to standardize access to biospecimens. The following paragraph, based on the recommendations developed by the National Cancer Institute of the United States of America (NCI), develops general principles to guide the procedure for access to specimens for research purposes (Office of Biorepositories and Biospecimens Research-NCI, 2007).

1. Although BRCs have the right to establish

priorities for access to specimens, in principle BRCs should commit themselves to providing equal right of access to researchers.

2. A mechanism of rapid peer and/or stakeholder review should be in place to set up priorities as to how collected specimens should be allocated to qualified recipient investigators. Preferably, this process is coordinated and chaired by the biological resource manager.

3. The proposed research project and use of specimen should be consistent with participants' consent, research purpose and permitted use of specimens.

4. During evaluation of the proposal it needs to be considered whether the proper expertise has been brought together in the research project and whether the lab is adequately equipped to perform the proposed experiment.

5. Within the above principles, the main criteria for approving requests for access should be:

- the scientific validity of the research proposal;
- the investigator's and institution's research qualifications;
- the investigator's written agreement covering confidentiality;
- use, disposition, and security of specimens and associated data;
- the investigator's written agreement in a Material Transfer Agreement covering publication, sharing of research results, and ownership of future intellectual property;
- ethical approval of the proposed research; and
- the funding level for the project.

Principles for international specimen exchanges

Many countries have adopted safeguard mechanisms and regulations to ensure the security of specimens and associated personal data as well as to protect the right of ownership and intellectual property that may stem from research conducted using biospecimens collected on their national territory. An international compilation of human subject protection has been compiled by the Office for Human Research Protections of the US Department of Health and Human Services (http://www.hhs.gov/ohrp/international/HSPCompilation.pdf). In several instances, however, these measures may tend to impose restrictions on international specimen exchanges, thus having a detrimental effect on developing large, multi-centric studies.

It is therefore important to develop international procedures to facilitate and oversee human specimen exchanges that respect the principles of national and international regulations on human subject research protections. Under such procedures, studies that meet a number of conditions may be granted a waiver of restrictions on specimen exchanges. Key conditions are listed below:

1. The study should be developed in the context of a scientific partnership between scientists and institutions of countries that are "specimen providers" and "specimen users"

2. The study must have been approved by the relevant institutional and legal ethical review boards in all the countries that are part to the study.

3. Personal and individual data accompanying the specimens should be anonymised.

4. Packaging, shipping and sending should comply with international regulations on the transport of hazardous specimens (see paragraph Specimen shipping)

5. The involvement of all parties should be regulated by a "Specimen Transfer Agreement" (STA) describing the nature of the involvement of each partner, the sharing of intellectual property and authorship, and the measures for proper re-storage or despatching of specimen leftovers.

6. Such Specimen Transfer Agreements should be guided by a principle of shared access to technology, knowledge, training and benefits of research.

7. When the specimen exchange involves

countries of different socio-economic status, it is fair to include in the STA provisions to ensure that any application deriving from the research performed using the specimens should be made available at costs compatible with the resources of the country with the lowest socio-economical status.

BRC work flow pattern

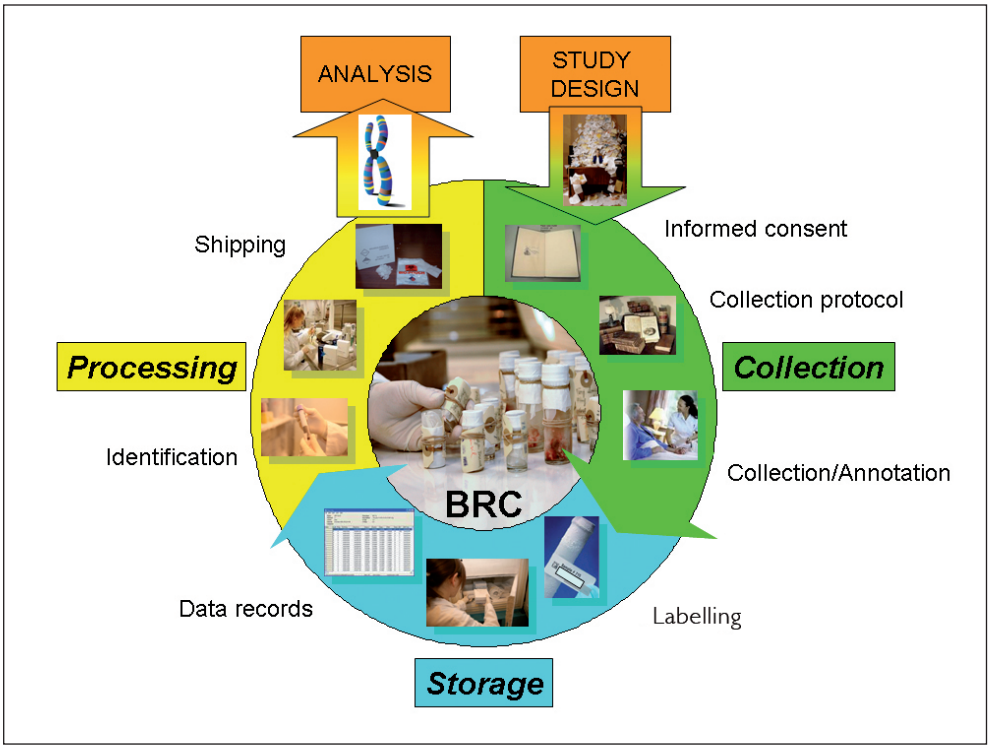

Figure 1 : BRC work flow pattern

Figure 1 shows the sequence and the flow of information, data and biospecimens, from the study design to final laboratory analyses. This scheme underlines the central role of BRC as the transfer structure between biospecimen collection and laboratory analysis. It also underlines the fact that, in developing a study protocol, each step in this sequence of events must be clearly defined. The flow of information and biospecimens, as defined by protocols and procedures, will ensure the constitution of a collection containing traceable biospecimens yielding interpretable results. The BRC is an essential source of information and recommendations for the collection of biospecimens, their annotation, storage, processing and flow from the participant to the laboratory where it will be analysed.

Selected protocols

Most of the following protocol guidelines are derived from the methods and protocols publicly available and were adapted or replaced according to comments of experienced bio-resource experts in the field. However, it must also be noted that these methods have been developed with a specific end use of specimens in mind (e.g. proteomics), and thus may not be suitable in all cases. Therefore these protocols are only given as indications.

Processing of blood specimens

If serum and plasma are being collected, it is important to prioritize the separation of them so they can be frozen as soon as possible. This is critical for time-sensitive samples for protein studies, for example. For processing of blood specimens we recommend to use the following protocols.

Filter papers

Always handle filter papers wearing gloves and only by the upper corner, marked out for labelling. Do not allow the card to come into contact with any unclean surface (e.g. bench, base of hood). Use EDTA/ACD tubes to produce filter papers.

1. Mix anticoagulated blood containing vacutainers by inversion before starting.
2. Wipe top of vacutainers with 70% ethanol before opening the lid.
3. Make 2 filter papers by placing 40µl of blood in the circle using a micropipette.
4. Air dry the filter papers thoroughly in the back of the Class II Biological Safety Cabinet.
5. Store filter papers in a paper envelope (not plastic) at room temperature.

Blood pellets (white cells)

Blood pellets can be used for the isolation of DNA (from EDTA / ACD tubes)

1. Transfer blood from the original tube to a labelled 50ml tube.
2. Fill tube with Tris-EDTA buffer (formula) and mix vigorously. Place on ice for 5 to 10 minutes.
3. Spin at 1200xg for 10 minutes.
4. Carefully pour off supernatant into a beaker containing chlorine bleach. Briefly vortex the pellet and add 50ml Tris-EDTA buffer. Shake vigorously.
5. If division of the sample is necessary, at this point pour 25ml of the sample into another falcon tube.
6. Spin both tubes at 1200xg for 10 minutes.
7. Repeat washing if red cells persist.
8. Carefully pour off supernatant.
9. Using a swirling motion, remove the pellet with a pipette and transfer to labelled cryovial.
10. Store in -80°C or liquid nitrogen until further use.

As an alternative, red cells can be lysed by using an ammonium-containing lysis buffer.

Plasma

Plasma collected in EDTA and ACD tubes, can be used for bioassays, plasma DNA isolation, proteomic analysis, and biomarker discovery.

1. Spin vacutainer (about 9ml) at 815xg for 10 minutes at 4°C to separate plasma from blood cells.
2. After wiping each tube with 70% alcohol, remove about 3ml plasma. Tube can be retained for white blood cell extraction.
3. Transfer to a labelled 15ml tube and centrifuge at 2500xg for 10 minutes at 4°C.
4. Aliquot plasma into 1ml labelled cryovials (3 to 4 aliquots).
5. Place in liquid nitrogen Dewar to snap freeze.
6. Store at -80°C or in liquid nitrogen.

The purpose of double spinning the plasma is to remove all cellular contaminants so that the plasma is suitable for plasma DNA analysis. It is extremely important, therefore, not to disturb the buffy coat after the first spin, and any pellet after the second spin.

Platelet-poor plasma

Platelet-poor plasma can be used for the isolation of plasma DNA (from EDTA tubes)

1. Spin blood at 3200xg for 12 minutes at room temperature.
2. Pipette off plasma using a plastic pasteur pipette. Transfer into tube.
3. Spin plasma at 2000xg for 10 minutes at 4°C.
4. Aliquot into 1ml aliquots in labelled cryovials.
5. Store at -80°C.

Serum

The blood is collected into tubes without addition of anticoagulants. Then two phases are distinguishable, a solid phase containing fibrin and cells, and a fluid phase containing the serum.
This process should be completed after 30 minutes at room temperature, after which the process described below starts.

1. Spin blood at 1500xg for 10 minutes at room temperature.
2. Aliquot 1ml portions of supernatant into labelled cryovials.
3. Place into liquid nitrogen Dewar or dry ice to snap freeze.
4. Transfer to -80°C freezer or liquid nitrogen.

White Blood Cells

White blood cells collected in EDTA and ACD tubes can be used for DNA extraction and the creation of cell lines.

1. Transfer the remaining blood from the plasma spin to a labelled 50ml tube containing 10ml RPMI 1640.
2. After alcohol swabbing the lid of this tube, aliquot 3ml Ficoll into each of two clearly labelled 15ml tubes.
3. Carefully layer 9ml diluted blood onto each tube of Ficoll. Treat gently, do not mix, but spin as soon as possible.
4. Spin at 450xg for 30 minutes. Note: when centrifuging, do not use brake.
5. Remove most of the top layer (RPMI 1640) using a 1ml Eppendorf tip and discard ≈ 3-4ml into waste container containing chlorine bleach.
6. Collect white blood cells with the same Eppendorf tip using a swirling motion to ‘vacuum up’ white blood

cells. Do not take too much Ficoll (third layer), as it is toxic to the cells. Place the white blood cells in a labelled 15ml tube containing 10ml RPMI.

7. Spin at 450xg for 10 minutes.

8. Pour off the supernatant into a waste container containing chlorine bleach. Add 3ml of cold freezing mix (10% DMSO, 20% FCS, RPMI 1640) and resuspend.

9. Dispense the white blood cells into 3 x 1ml labelled cryovials that have been sitting on ice.

10. Place on ice. Place vials in a rate-limiting freezer as to cryopreserve cells in conditions that maintain cell viability. This should be done as soon as possible as DMSO is toxic at room temperature.

11. Transfer on a weekly basis to liquid nitrogen tanks.

Instead of a separation based on Ficoll, a Percoll separation can be used alternatively.

Buffy coat cells

The buffy coat is a thin, greyish-white layer of white blood cells (leukocytes and lymphocytes) and platelets covering the top of the packed red blood cells after 450xg centrifugation (from EDTA/ACD containing blood tubes).

1. After having spun the blood, take buffy coat off with about 100µl of plasma using a disposable sterile Pasteur pipette: be careful not to lift red cells.
2. Lyse remaining red cells by addition of red cell lysis buffer at room temperature.
3. Spin tube at 450xg for 10 minutes at room temperature.
4. Resuspend the pellet.
5. Aliquot as appropriate into labelled cryovials.
6. Place in liquid nitrogen to snap freeze.
7. Store in liquid nitrogen.

Whole blood

To be prepared from EDTA tubes. The anti-coagulated blood can be snap frozen as it is. In case the blood cells are needed intact, DMSO is needed to keep them alive while freezing.

1. Dispense 50µl DMSO into two 1ml sterile cryovials.
2. Invert EDTA tube twice then add 450µl of blood to each cryovial.
3. Invert cryovial to mix the whole blood with the DMSO. Note: DMSO is cytotoxic at room temperature; therefore as soon as it is mixed with blood, it should be placed in a controlled-rate freezer.
4. Transfer to -80°C after at least 4 hours.

Processing of solid tissue specimens

Careful and well-documented processing of tissue specimens is crucial to the overall usefulness of the repository as a resource for scientific research. This protocol for collecting and freezing tissue samples was developed within TuBaFrost, a European project aimed at producing a European virtual frozen tumour bank (TUBAFROST, 2003). We recommend using this protocol which contains choices and recommendations for preserving solid tissue.

Snap-freezing

Safety

All procedures should be carried out in accordance with the local codes of practice. Working with liquid nitrogen and isopentane is hazardous—all procedures must comply with local safety rules specific to these chemicals. All tissue must be handled as if potentially infectious.

Collection of tissue

Ward

Consent must be obtained from patient before surgery (if applicable, according to the law in the collecting country)

Operating theatre

Deliver notification of tissue collection (and consent form if needed) to surgeon or flag up on operating list.

Surgeon

1. Complete pathology form (if possible in advance).
2. Perform operative procedure, record time of excision of specimen.
3. Place specimen in labelled sterile pot/bag and put on ice.

Operating theatre staff

Send fresh tissue specimen immediately to pathology department.

Histology Departement

1. Notify pathologist and tissue bank research technician (if not already present).
2. Check paperwork and allocate pathology number to specimen as routine.

Pathologist

1. Macroscopically describe specimen as usual.
2. Using clean instruments and on a clean surface (sterile foil or clean dissection board), dissect the tissue specimen. Clean or change instruments between dissecting normal and tumour tissue.
3. Take representative parts of tissue for routine diagnosis (for fixation and embedding) as priority and decide if there is sufficient material available for the tissue bank.
4. Supply research technician with tissue sample(s) for cryostorage; representative parts of the lesion, normal tissue and pre-malignant conditions.

Technician

1. Prepare the tissue sample for snap-freezing on a clean surface and using clean instruments—change instruments between preparing normal and tumour tissue. The minimum size of tissue for snap freezing is approximately 0.5cm3, though the amount of tissue available will differ depending upon the sample site. Smaller fragments should still be snap-frozen and stored in the tissue bank. If there is sufficient material, freeze duplicate samples.

2. Pre-cool the freezing medium isopentane (2-methyl butane) to the moment when opaque drops begin to appear in the isopentane and the solution becomes misty; this will bring the isopentane towards its freezing point (-160°C), the optimal freezing point for the tissue. Options:
A. LN2: suspend a vessel of isopentane in LN2
B. Dry ice: add dry ice (cardice) to the isopentane until a slush is formed, or by suspending a vessel of isopentane in dry ice.

3. Label cryovials, cryomolds or cryostraws with a barcode and/or sequential code (depending upon local laboratory practice). Use a waterproof pen able to withstand long-term storage at low temperatures. The sequential code is the local inventory code and must not relate to the pathology number or other identifiers. If a barcode is used, readable recognition must also be included to make the sample identifier readable at institutions where there are no barcode readers.

4. Record the local sequential code, pathology number, date, lag time from excision to freezing, and the type of tissue (site and whether the sample is tumour/normal/premalignant) in the inventory book. If a barcode system is in use, this can be scanned into the Laboratory Information Management System and the above data recorded.

5. Freeze directly in isopentane. Do not remove the tissue from the isopentane until freezing is complete (only 5 seconds or less is needed depending on size) but ensure sample does not crack. Remove sample from isopentane and enclose in the labelled cryovial. Strive to snap freeze all tissue within 30 minutes of excision from patient. Tissue subject to a delay of up to 2 hours should still be collected and the delay noted within the local inventory database. Options for freezing:
A. Embed the tissue samples in optimal cutting temperature (OCT) compound and freeze in isopentane or freeze directly in isopentane. The isopentane used is either cooled by suspension in liquid nitrogen or through addition of dry ice. Do not remove the tissue from the isopentane until freezing is complete (5 seconds or less depending on size) but ensure sample does not crack. Remove sample from isopentane and enclose in the labelled cryovial.
B. Orientate the tissue on a piece of cork and an equally sized piece of Whatman paper soaked in physiologic salt solution. The isopentane used is either cooled by suspension in liquid nitrogen or through addition of dry ice. Do not remove the tissue from the isopentane until freezing is complete (5 seconds or less depending on size) but ensure sample does not crack. Remove sample from isopentane and enclose in the appropriate labelled storage vessel.
C. Embed samples in a cryosolidifiable medium in plastic cryomolds and immerse in the pre-cooled isopentane. The isopentane used is either cooled by suspension in liquid nitrogen or through addition of dry ice. Do not remove the tissue from the isopentane until freezing is complete (5 seconds or less depending on size) but ensure sample does not crack.
D. If the cryostraw system is used introduce a carrot of tissue into the straw, thermically seal each extremity and place in liquid nitrogen.

Storage of tissue

Storage of tissue can be done according different protocols according the equipment available within the facility. Options for storage:

A. Transfer the snap-frozen sample from the isopentane to a pre-chilled storage container for transfer to either a locked –80°C freezer or liquid nitrogen storage facility in liquid or vapour phase. For storage longer than 5 years, liquid nitrogen is recommended.

B. Place cryostraws in a designated visotube within a goblet (removable liquid nitrogen storage elements) and place within the locked liquid nitrogen repository.
B1. Store duplicate samples in a different storage facility if this is available.
B2. Check the back-up system for the storage repository—either a back-up freezer running constantly or adequate supplies of liquid nitrogen.
B3. Record storage details in the inventory book and check earlier data that were entered. At a minimum the information recorded will include: inventory number (local sequential code), location, pathology number, type of tissue (site and also whether the sample is tumour/unaffected (normal)/premalignant), lag time between excision and freezing, and date.
B4. Transfer details to the computerized database system.
B5. Update the database when samples are moved or depleted.

Formalin Fixation

Formalin fixation is standard practice in most routine histopathology laboratories. The following guidelines address specific issues related to preservation of formalin-fixed specimens in BRCs.

1. Tissue specimens should not be bigger than 1.5 x 1 x 0.5cm.
2. Specimens will be fixed in fresh 10% neutral buffered formalin (NBF) Blue for a minimum of 4 and a maximum of 48 hours, after which time they will be embedded in paraffin following conventional techniques.
3. All reagents should be DNAse- and RNAse-free (e.g. prepared using DEPC water).
4. Fixation media such as Bouin's containing picric acid should be avoided, as this compound interferes with subsequent PCR analysis of extracted nucleic acids.
5. Alcohol fixation may be used as an alternative to formalin. For this, tissue is placed into 70% alcohol (diluted with DEPC water) for a minimum of 4 hours.

Alternatives for formalin can be desirable to use as a routine fixative, due to the chemical hazards of formalin. The effect on long-term storage using these alternative fixatives on the desired macromolecules is not always known, however, and should be empirically established.

RNA*later*

This substance protects RNA in fresh specimens. It eliminates the need to immediately process or freeze samples.

Tissue

Cut tissue to be less than 0.5cm in at least one dimension, then submerge tissue in 5 volumes of RNA*later* (e.g. a 0.5g sample requires about 2.5ml of RNA*later*).

Cells

Resuspend pelleted cells in a small volume of PBS before adding 5—10 volumes of RNA*later*.

Storage

RNA*later*-treated tissue and cell samples can be stored at 4°C for one month, at 25°C for one week or at -20°C for indefinite time. For RNA isolation, simply remove the tissue from RNA*later* and process.

Processing of urine and buccal cells

The following protocols for processing of urine and buccal cells contain recommended procedures.

Urine

1. Plastic or glass containers for collection of urine should be clean and dry, have a 50–3000ml capacity, a wide mouth and leak-proof cap, and should be clearly labelled.
2. When in transit, urine collections should be maintained on ice or refrigerated.
3. Urine should be aliquoted according to the volume necessary for analysis or storage.
4. Depending on the analyte to be measured, a preservative may be added during collection or before aliquoting.
5. Store urine at -80°C or lower in liquid nitrogen.

Buccal Cell

1. A collection kit (containing mouthwash, 50ml plastic tube, plastic biohazard bottle, and courier packaging) is mailed or given to the participant, along with an instruction sheet. The participant is to brush the teeth as usual, rinse the mouth well twice with water, and then wait 2 hours. They should not eat or drink anything other than water during this time.
2. After 2 hours, 10ml of commercial mouthwash should be poured into the tube, and then 10ml tap water added. This diluted mouthwash should be placed into the mouth (without swallowing) and swished around vigorously for 30 seconds.
3. The mouthwash should then be spat back into the plastic tube, and the tube should be sealed tightly.
4. The sample should be sent back to BRC immediately for processing, or stored at 4°C until sent but should be sent within 24 hours.
5. On arrival at the laboratory, transfer mouthwash to 15ml conical test tubes.
6. Add 35ml Tris-EDTA to the mouthwash sample and spin at 450xg for 5 minutes.
7. Decant supernatant and discard.
8. Wash cells twice, each time with 45ml Tris-EDTA.
9. Resuspend cell pellet in 50µl Tris-EDTA and transfer to 2ml labelled cryovials.
10. Store sample at -80°C or in liquid nitrogen.

Note: Buccal cells can also be collected with other means such as brushes.

Saliva

A research consortium at the University of California-Los Angeles was funded by the National Institute of Dental and Craniofacial Research (NIH grant UO1 DE 16275; PI: David T. Wong DMD, DMSc) to investigate the human saliva proteome. The protocol for saliva collection and processing is derived from their “Salivary Proteome Handbook Procedures and Protocols”. (National Consortium for the human saliva proteome, 2007)

Collecting and Processing Saliva

1. Saliva collection is recommended to be done in the morning (please aim for 10-11am if possible). Ask the subject to refrain from eating, drinking or oral hygiene procedures for at least 1 hour prior to the collection.
2. The subject should be given drinking water (bottled) and asked to rinse their mouth out well (without drinking the water).
3. Five minutes after this oral rinse, the subject should be asked to spit whole saliva (WS) into a 50ml sterile Falcon® tube.

The subjects need to refrain from talking. It is better for subjects to drop down the head and let the saliva run naturally to the front of the mouth, hold for a while and spit into the tube provided. Subjects will spit into the collection tube about once a minute for up to 10 minutes. The goal for each whole saliva donation should be about 5ml. Require that the tube be placed on ice while collecting whole saliva. Remind the subjects not to cough up mucus as saliva is collected, not phlegm.

4. Submandibular saliva (SM) collection: use 2 x 2 inch cotton gauze to block the opening of each parotid duct; dry up the floor of the mouth and block the openings of the sublingual gland (both sides) and have the subject raise their tongue slightly to elevate the opening to the SM gland; begin to collect SM saliva by using a sterilized Wolf device. A sterilized and disposable yellow tip (for pipette P200) is connected into the device and changed between every collection. During the collection, at 2 minute intervals, a few grains of citric acid powder are swabbed with a moistened cotton applicator onto the lateral dorsum of the tongue to stimulate the secretion. Aim to collect at least 200µL SM.
5. Sublingual saliva (SL) collection: similar to the protocol described above for SM collection. The only difference is that the ductal orifices of the submandibular gland will be blocked off. Aim to collect >100µL

SL every time.
6. Parotid saliva (PR) collection: use a parotid cup to collect PR. Parotid cups may be placed bilaterally if the clinical investigator so chooses. This will allow for the simultaneous collection from each parotid gland. The citric acid stimulation will be performed as described above. Aim to collect >1mL PR. The first 0.1ml of parotid saliva collected will be discarded to insure that fresh parotid saliva is obtained.
NOTE: COLLECTED SAMPLES ARE TO BE KEPT ON ICE AT ALL TIMES PRIOR TO PROCESSING.
7. Sample processing using proteinase inhibitors: To each 100µl saliva, add:
   a) 0.2µL proteinase inhibitor cocktail from standard stock solution (Sigma, cat# P8340), invert gently.
   b) 0.3µL Na3OV4 (Sigma, cat# S6508) from standard stock of 400mM, invert gently.
8. Centrifuge specimens at 2600xg for 15 minutes at 4°C; (if you note that incomplete separation has occurred, increase the spin time to 20 minutes).
Remove the supernatants from the samples and mark them as plus "super" which stands for the supernatant phase of the saliva. Taking care not to disturb the pellet and keeping the pellet as is in the original tubes, mark the original tubes with "pellet".
9. Freeze samples at -80°C.

Processing of cervical cells

In a Pap smear test, a sample of cells is taken from the uterine cervix using a spatula or brush, smeared onto a slide, and examined under a microscope for abnormal cells (precancer or cancer). This protocol is a selected protocol from diverse collection procedures.

Note the following:
1. It is best not to take a smear from women who are actively menstruating or have symptoms of an acute infection. Slight bleeding is acceptable.
2. Pregnancy is not an ideal time for a Pap smear, because it can give misleading results.

Taking the smear with a wooden spatula or a brush
1. Insert the long tip of the spatula into the cervical os, and rotate it through a full circle (360 degrees). If the cervix broom brush is used, the tip of the brush is placed within the cervical os and then rotated gently for three 360° circles.

Taking a Pap smear
2. Smear both sides of the spatula (or the contents of the brush) onto the glass slide with one or two careful swipes. If you see any abnormalities outside the area sampled, take a separate specimen and smear it on another slide.
3. Immediately fix each slide. Either use spray fixative, at a right angle to, and a distance of 20cm from, the slide, or immerse the slide in a container of 95% ethanol for at least 5 minutes. If the slide is not fixed immediately, the cells will dry and become misshapen; it will then not be possible to read the slide accurately in the laboratory.
4. Gently close and remove the speculum.
5. Place all used instruments in decontamination solution.

After taking the smear
6. Label the frosted edge of each slide carefully
7. On the patient record, note and illustrate any features you have noted: visibility of the transformation zone, inflammation, ulcers or other lesions, or abnormal discharge. Note whether other samples were taken, for example Pap smear of other areas and, if the woman has been referred elsewhere, to whom and when.

Processing of hair and nails

These protocols are recommended for collecting hair or nails specimen.

Hair

Head hair may be collected as follows:

1. Along an imaginary line drawn across the middle of the back of the head from the centre of one ear to the centre of the other, gather a lock of hair at least the thickness of a pencil, and tie it together near the root end (near the scalp) using a small string or a rubber band.
2. Cut the hair as close to the scalp as possible without cutting the scalp.
3. Maintain the horizontal position of the hairs in the bundle by wrapping the cut section in aluminium foil or plastic wrap.
4. Indicate the root-end and tip-end by marking the foil or plastic wrap with a permanent marker or with a paper label. **Do not use tape on the hair itself.**
5. Place the specimen in a clean, dry, labelled paper envelope for shipment to the laboratory. Note whether bleaches, hair dye or medications (e.g. selenium or minoxidil) were used.

Please note that hair from other sources (pubic, axillary, beard, moustache, chest, etc.) may also be analyzed if head hair is not available (NMS labs).

Nails

A clean pair of nail clippers is to be used. To clean thoroughly, nail clippers are rubbed with alcohol swabs. Nails should be clean of all polish, dirt and debris. Nail clippings from each finger or toe should be collected and packaged separately in plastic bottles. Each bottle should be labelled with the mass of the nail collected and its source, e.g., right index finger (NMS labs and Expertox).

Abbreviations

BRC	Biological Resource Centre
DGR	Dangerous Goods Regulations
DNA	DeoxyriboNucleic Acid
EORTC	European Organisation for Research and Treatment of Cancer
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IARC	International Agency for Research on Cancer
IATA	International Air Transport Association
ICAO	International Civil Aviation Organization
ICD-O	International Classification of Diseases for Oncology
ISBER	International Society for Biological and Environmental Repositories
LIMS	Laboratory Information Management System
LN2	Liquid Nitrogen
MRC	Medical Research Council
MTA	Material Transfer Agreement
NCI	National Cancer Institute
OCT	Optimum Cutting Temperature
OECD	Organisation for Economic Co-operation and Development
OECI	Organisation of European Cancer Institutes
PCR	Polymerase Chain Reaction
QA/QC	Quality Assurance/Quality Control
R&D	Research and Development
RNA	RiboNucleic Acid
SOP	Standard Operating Procedures
TNM	TNM classification of malignant tumours
TUBAFROST	The European Human Tumor Frozen Tissue Bank
UNECE	United Nations Economic Commission for Europe
WHO	World Health Organization

Glossary

Biorepository: a place, room or container where biospecimens are stored. Biorepositories vary considerably, ranging form formal organisations to informal collections of materials in an individual researcher's freezer.

Biospecimen: a quantity of tissue, blood, urine or other biologically derived material used for diagnosis and analysis. A single biopsy may generate several biospecimens, including multiple paraffin blocks or frozen biospecimens. A biospecimen can include everything from subcellular structures (DNA) to cells, tissue (bone, muscle, connective tissue and skin), organs (e.g. liver, bladder, heart, kidney), blood, gametes (sperm and ova), embryos, fetal tissue, and waste (urine, faeces, sweat, hair and nail clippings, shed epithelial cells and placenta).

Collection: assemblage, for research purposes, of biological material selected on the basis of clinical or biological characteristics.

Dry ice: solid phase carbon dioxide.

ICDO: the International Classification of Diseases for Oncology (ICD-O) is a domain specific extension of the International Statistical Classification of Diseases and Related Health Problems for tumour diseases. This classification is widely used by cancer registries.

Informed consent: an educational process between the investigator and the prospective subject (or the subject's legally authorized representative) as a means to ensure respect for persons; mutual understanding of research procedures, risks, rights, and responsibilities; and continuous voluntary participation.

Material Transfer Agreement: a binding label agreement between the provider of research materials and the recipient of the materials, setting forth conditions of transfer and use, protecting proprietary interests, and restricting distribution of the material. An important aspect of the MTA is that it normally removes liability on the part of the provider that might arise from the recipient's use of the research material.

Patient: a person undergoing medical treatment.

Processing: any procedure employed after biospecimen collection but prior to its distribution, including preparation, testing, and releasing the biospecimen to inventory and labelling.

Quality assurance: an integrated system of management activities involving planning, implementation, documentation, assessment and improvement to ensure that a process or item is of the type and quality needed for the project.

Quality control: specific tests defined by the Quality assurance to be performed to monitor procurement, processing, preservation and storage, biospecimen quality, and test accuracy. These may include but are not limited to performance evaluations, testing and controls used to determine accuracy and reliability of the biorepository's equipment and operational procedures as well as monitoring of the supplies, reagents, equipment and facilities.

Sample: portions of biospecimens distributed to researchers.

Subject: a living individual about whom an investigator, either professional or student, conducting research obtains (1) data through intervention or interaction with the individual or (2) identifiable private information. A human subject may also be a patient, but is not necessarily one.

Tissue: refers generally to a biologic collection of cells, and the extracellular matrix and/or intercellular substances surrounding them. Tissue is most often referred to in the context of solid tissue, as originating from a solid organ; however, tissue can also be defined more broadly to include collections of cells and intercellular substances from bodily fluids such as blood.

TNM Classification of Malignant Tumours: TNM is the cancer staging system developed and maintained by the International Union Against Cancer (UICC) to achieve consensus on one globally recognised standard for classifying the extent of spread of cancer. The Mandatory parameters ('T', 'N', and 'M') are:

T ((0),1-4): size or direct extent of the primary tumour

N (0-3): spread to regional lymph nodes

M (0/1): distant metastasis

Use of an «X» instead of a number or other suffix means that the parameter is not assessed.

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