## 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).*

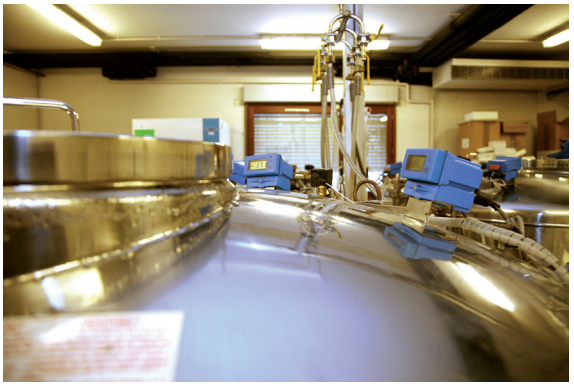

**Figure 1**

**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.

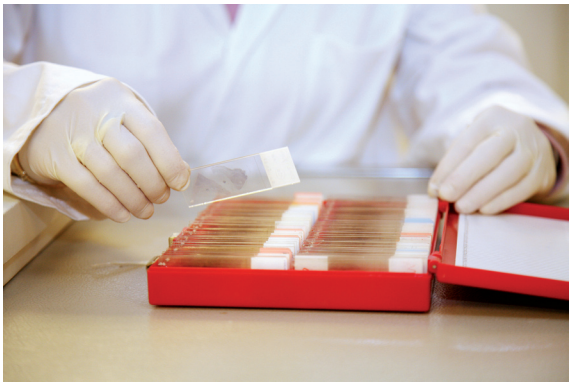

**Figure 7**

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).*

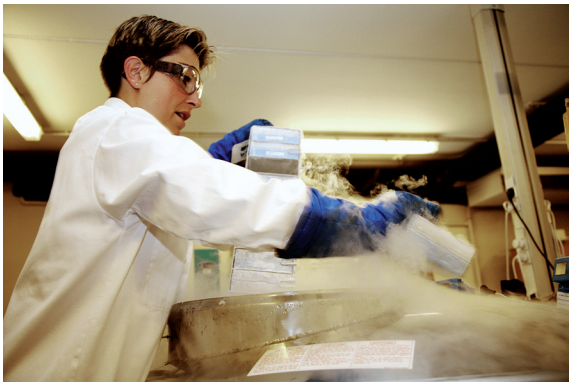

**Figure 9**

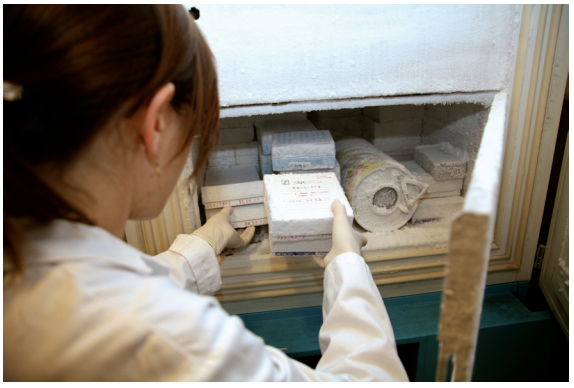

**Figure 10**

**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).*

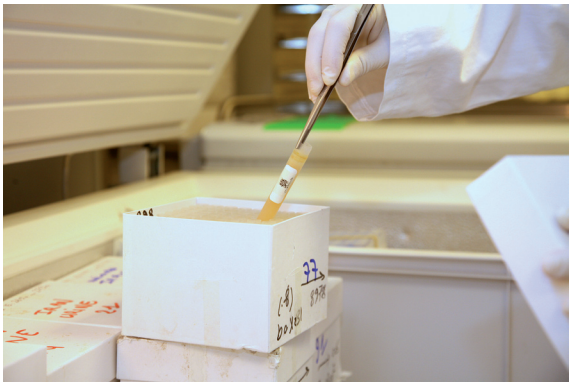

**Figure 12**

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)*

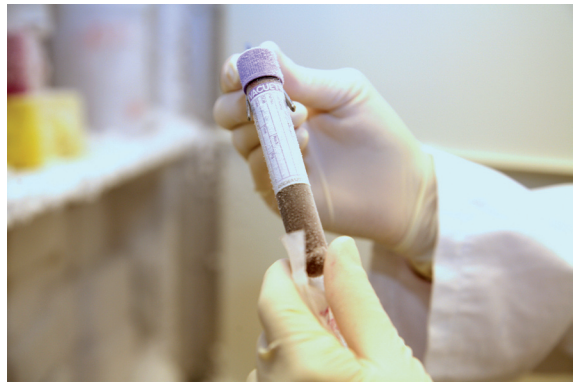

**Figure 13**

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).

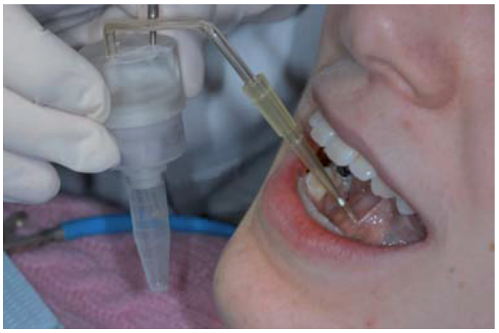

**Figure 16**

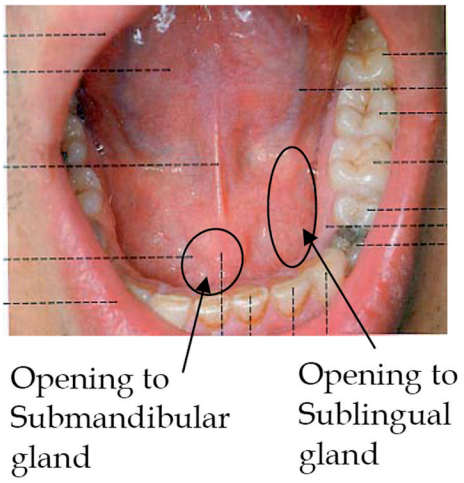


**Figure 17**

*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)*

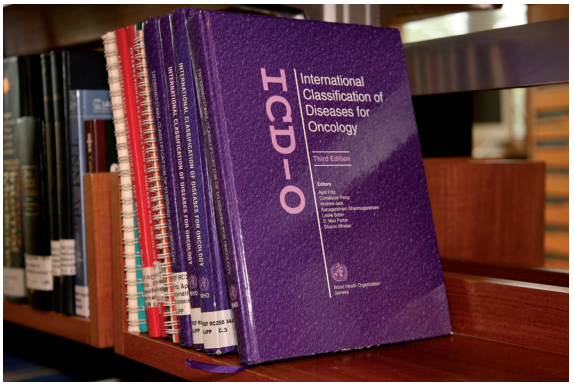


**Figure 18**

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

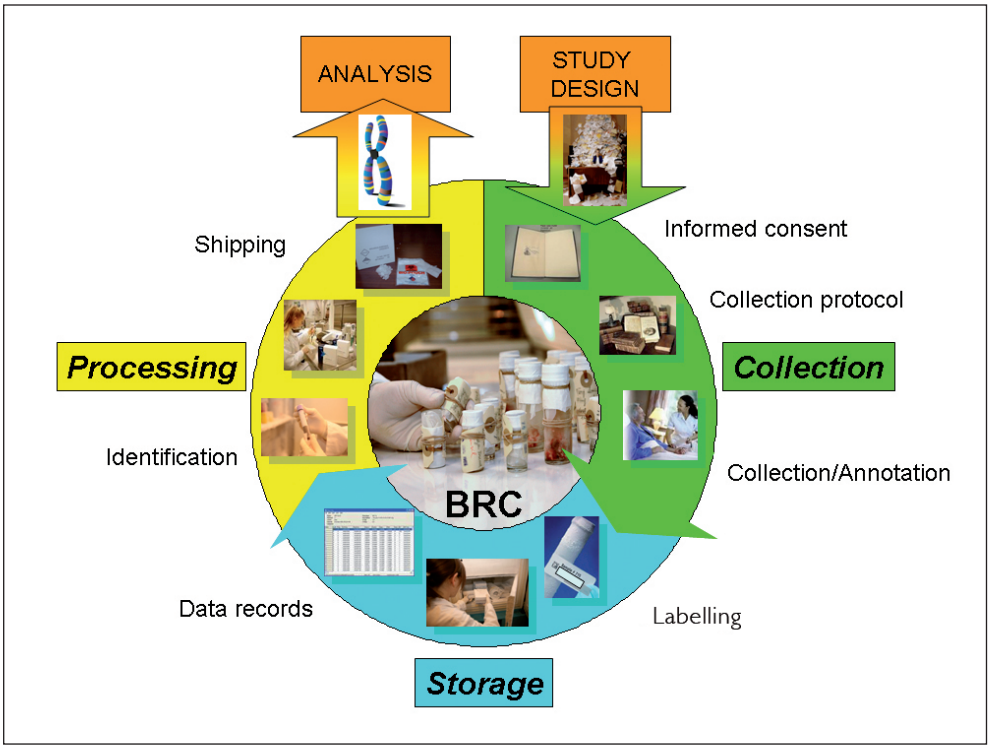


**Figure 1 : BRC work flow pattern**

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.