Chapter 1

Organizational Issues in Providing High-Quality Human Tissues and Clinical Information for the Support of Biomedical Research

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Summary

Superior-quality human tissues are required to support many types of biomedical research. To be useful optimally in supporting research, not only must these tissues be accurately diagnosed, but also the specific aliquots of tissue supplied to investigators must be accurately described as part of the quality control analysis of the tissue. Tissues should be collected, processed, and stored uniformly. Some tissues are provided to investigators from tissue banks for which tissues have been collected and processed according to standard operating procedures (SOPs) of the tissue bank. Other tissues provided to support research are collected and processed according to SOPs modified to meet investigator needs and requirements, i.e., prospective collection/processing. These different models of tissue collection require different goals, designs, and SOPs. The objectives of tissue repositories also vary based on the types of tissues provided (e.g., fresh tissue aliquots, fixed paraffin-embedded tissue, paraffin tissue sections, etc.) and how the tissues are to be used in research. For example, the potential use of tissues affects the need for extensive annotation of the specimen including both clinical information (e.g., clinical outcomes) and demographics. Specifically, if the tissues are to be used for extraction of proteins or basic studies of disease processes, less clinical information, if any, may be needed than if the tissues are to be used for the correlation of an aspect of the disease process with clinical outcome or response to a specific therapy. In this review, we describe, based on our experience, the major issues that should be addressed in designing and establishing a tissue repository.

Key words: Human tissue, Tissue banking, Tissue repository, Research infrastructure, IRB, HIPAA

Abbreviations

CHTN	Cooperative Human Tissue Network
DCIS	Ductal carcinoma in situ
DMSO	Dimethyl sulfoxide
GMP	Good manufacturing practices
HIPAA	Health Insurance Portability and Accountability Act
ISBER	International Society for Biological and Environmental Repositories
ISO	International Organization for Standardization

Robert Grützmann and Christian Pilarsky (eds.), *Cancer Gene Profiling: Methods and Protocols*,
Methods in Molecular Biology, vol. 576,
DOI 10.1007/978-1-59745-545-9_1,

LCIS	Lobular carcinoma in situ
LN_2	Liquid nitrogen
NCI	National Cancer Institute
OCT	Optimal cutting temperature (compound for embedding specimens prior to cryosectioning)
PHI	Protected health care information
PSA	Prostatic-specific antigen
QA	Quality assurance/management
QC	Quality control
SOP	Standard operating procedure

1. Introduction

Modern biomedical research requires access to superior-quality specimens of human tissue and bodily fluids with or without extensive clinical annotation *(1–2)*. Different types of organizations devoted to supplying tissues for research have varying goals selected to meet different tissue and informational needs *(3)*. In this review, we discuss multiple models of tissue repositories and, based on our experience, several of the more important issues affecting the design and operations of a tissue repository. A detailed discussion of most of these issues is beyond the scope of this chapter. Thus, we have referenced articles that provide additional information on these topics *(1–19)*. We have also provided examples of standard operating procedures (SOPs); one for the processing of blood and another for the processing of tissue.

2. Models of Tissue Collection

Obtaining human tissues and bodily fluids to support biomedical research may utilize an organized or disorganized approach. "Catch as catch can" is the best designation of the approach in which a surgeon, pathologist, or other medical personnel provides tissues to investigators via an unorganized approach. Such specimens characteristically have not been collected, processed, or stored using SOPs and usually are not associated with quality control; thus, these specimens may be of poor quality and their diagnosis may be incorrect. More worrisome is that such specimens may be obtained without oversight of Privacy Boards or Institutional Review Boards (IRBs) and may violate the Common Rule and/or the Health Insurance Portability and Accountability Act (HIPAA) regulations.

2.1. Prospective Collection

An organized approach in which investigators specify exactly the tissue specimen they need as well as how the specimens are to be processed and stored is designated as the "prospective collection model." The clear disadvantage of prospective collection versus a banking model is that large numbers of specimens are not readily available immediately when requested. In addition, outcome data are not available because the specimens are collected when requested, so the patients' clinical outcomes may take several years to develop after collection. The advantage of the prospective collection model is that the investigator receives exactly what is requested (e.g., fresh uninvolved kidney minced in RPMI media). The investigator must, however, wait for specimen availability (weeks to months) and, when needed, on clinical outcome, which may take years.

2.2. Banking

Another approach to obtaining superior human tissues to support biomedical research is to utilize a "banking model." In a banking model, SOPs are followed for obtaining, processing, and storing human tissues. For example, a bank may store only frozen tissues and/or paraffin blocks. "Specially processed" tissues as well as fresh, unfrozen samples usually will not be available from such a bank. One of the major disadvantages is that the specimens may not meet specific requirements of the investigator for specific parameters such as aliquot size, percentage of tumor, and processing and/or storage methods *(3)*. Advantages of the "banking model" are that large numbers of specimens may be immediately available and clinical and demographic information including clinical outcome also are readily available.

2.3. Specimens Associated with Clinical Trials

A "clinical trial model" is a type of banking model in which the remnants of the tissues/bodily fluids collected from one or more clinical trials are banked to support future studies. The problems with this specific banking model compared with a general banking model may be magnified in that the original consent form of the clinical trial may not clearly state that the specimens can be used for research in addition to the clinical trial. Similarly, the institution's IRB may prohibit the utilization of specimens for a different type of research. In addition, the remnants of the clinical study may not meet the needs of a wide range of investigators, and remnant tissues may not be available from all original patients of the clinical trial.

2.4. Combination

The "tissue repository model" uses a combination of the approaches of the prospective and banking models, including the advantages of each of the models. The main potential problem in the operation of a tissue repository is that there are complex and numerous administrative requirements as well as the need for a more complex bioinformatics system. This chapter focuses primarily on a tissue repository model.

3. Matching Tissues to Tissue Requirements

3.1. Identification of Specimens

Correct identification of specimens is of critical importance to providing superior-quality tissues to support biomedical research *(3, 6, 12, 16, 17)*. A labeling method should be used that (1) minimizes the label separating from the specimen, (2) prevents mislabeling due to errors by personnel, and (3) avoids problems with reading the specimen identification (e.g., poor handwriting). For most tissue repositories, this is best accomplished by the use of bar codes that link the specimen to a database containing pertinent clinical, demographic, and historical information regarding the individual who was the source of the specimen. It should be understood that a bar code is only a "number" and this number contains no other information; it is only via the link of the number of the bar code to software that information regarding the bar-coded specimen is actually identified. Thus, unless the identical software is used at a second site, the second site can only read the specimen number and does not have access to a link with the information in the database. Any other information on the printed label, e.g., race, age, etc., comes from the software via the bar code and not directly from the bar code number.

3.2. Difficult to Fulfill Requests

Requests for very specific tissues become more difficult to meet as more requirements are placed on the request *(3, 16)*. Obviously, a request for any breast carcinoma is less difficult to supply than a request for well-differentiated breast carcinoma from an African American man younger than 40 years of age, because breast cancers are rare in males and in relatively young individuals. Another investigator requirement that makes a request difficult to meet is a request for very large amounts of tissue (e.g., 5 g) from tumors that are typically small. This includes tumors of the breast and prostate, which are usually small due to screening methods. Because some cancers (e.g., breast, prostate, pancreas) are in great demand by investigators, requests from multiple investigators, each requiring a small amount of tissue (e.g., 0.1 g), will more likely be filled than will a request for a large amount of tissue from these tumors *(3, 16)*. Similarly, for tumors in high demand, such as prostate or pancreas tumors, which tend to be small, requests for large numbers of cases within 1 year (e.g., 100) are unlikely to be met. Requests for large numbers of relatively rare tumors or tumors or other tissues that are not typically removed surgically cannot be provided (*see* **Subheading 4.2.4**). Most tissue repositories try to provide tissues equitably among those investigators requesting the same tissues. Because efforts (time) devoted to supplying specific tissue requests must also be divided equitably, tissue requests requiring extensive effort (e.g., removal of a vertebral column from a body or processing hundreds of

specimens by a complex protocol) cannot easily be met by a busy tissue repository. As discussed in **Subheading 3.4**, it is important for tissue repositories to educate investigators regarding which of their requirements make their requests difficult or impossible to meet. This includes unreasonable times for processing and freezing after the specimen is removed surgically (as discussed in **Subheading 3.3**).

3.3. Time Interval Between Surgery and Tissue Storage

In general, remnant diagnostic tissues should be reviewed by a pathologist or their designate to assure that the diagnostic integrity of the specimen is uncompromised. Unreasonable requirements of investigators for rapid processing and freezing of samples after surgery will reduce the availability of tissue to investigators for several reasons. Freezing samples in the operating room within minutes of removal from the patient may jeopardize the ability of a pathologist to review the material to ensure that it is not required for diagnosis. In addition, although some aliquots of tissue may be collected, processed, and frozen within 15 min of surgical removal, this usually requires special dedicated personnel and resources that many tissue repositories may not have. It is, however, important to record the time intervals between the removal of operative specimens or the transfer of these specimens from the operating room to the tissue repository, and these intervals should be minimized as much as possible.

After specimens are removed from patients, they should be maintained unfrozen, at approximately 4°C rather than at room temperature, while awaiting diagnostic review. Specimens provided for research should then be processed as rapidly as practicable; however, delays in processing may occur when multiple specimens must be processed simultaneously. In such cases, one or two aliquots from each specimen could be rapidly snap frozen in liquid nitrogen (LN_2) vapor and other aliquots could be collected and subsequently frozen.

The scientific importance of rapid collection and processing of tissue after a long period of warm ischemia in vivo (i.e., while blood vessels are compromised during surgery, *see* **Subheading 4.2.3**) is controversial. This is because many molecules will be affected by enzymes that function optimally at body temperature of warm ischemia. Thus, numerous molecular changes may occur before operative tissues are removed from the body. Huang et al. *(20)* evaluated the effects of in vitro ischemia on 2,400 genes in human tissue specimens using spotted arrays and found that less than 14% of the genes changed by more than 50%. Most genes at the messenger RNA (mRNA) level showed relatively modest increases (5 min after surgery versus 60 min after surgery). Similarly, Dash et al. *(21)* reported that less than 1% of genes demonstrated changes after 1 h of removal of prostate tissue from the body. In addition, Spruessel et al. *(22)* reported that 80% of genes

changed less than twofold within 30 min after removal from the body. Based on these studies of mRNA, very rapid removal (<30 min) to maintain nondegraded mRNA may not be justified.

Phosphoproteins are of special interest to molecular stability. Baker et al. *(23)* reported that 9 of 13 biopsies of adenocarcinomas of the esophagus expressed pAkt; however, pAkt was identified in none of the matching resected specimens. In addition, using a xenograft model, they reported a 180 min half-life of Akt and a 20 min half-life of pAkt. In contrast, Ayala et al. *(24)* detected pAkt in formalin-fixed, paraffin-embedded tissue from radical prostatectomies and used its presence/absence as a prognostic biomarker. Thus, phosphoprotein molecules may require rapid tissue processing. Nevertheless, this area remains controversial.

3.4. Education of Tissue Repository Personnel and Repository Users

Providing consistent and hence uniformly collected, processed, and stored tissues requires using SOPs in the operation of the tissue repository. All tissue procurement personnel need to be trained in all aspects of repository operations, including meticulous adherence to SOPs *(12, 17, 18)*. Training in safety as well as regulatory and ethical issues (keeping patient information confidential) also is very important, not only for personnel of the repository, but also for all users of the repository *(3, 16)*.

The repository should also serve as an educational resource for investigators and other clients. Clients frequently require assistance in selecting the optimal tissues to support their research. For example, clients need to understand why and how restrictive requirements on the tissues they request may prevent them from receiving tissues **(Subheadings 3.2** and **3.3)**. Similarly, they may need to understand repository limitations in collecting and processing tissues as well as how tissues differ in their appropriateness to support a specific research project (e.g., smooth muscle from the wall of the colon is different from smooth muscle of the uterus). All requests for human tissues should be reviewed by the pathologist or other equivalently knowledgeable professional associated with the tissue repository and, if necessary, guidance should be provided to the investigator concerning the appropriateness of the request for specific tissues. If a request is difficult to meet because of unnecessary requirements, this offers an excellent opportunity to explain to the requesting investigator limitations in tissue collection and processing.

3.5. Types of Tissues Collected and Services Provided

Tissue repositories may provide an array of tissues to investigators, ranging from paraffin sections of one type of cancer (e.g., breast) to fresh, frozen, and fixed solid tissues and bodily fluids from patients with a variety of disease processes both neoplastic and nonneoplastic. One of the initial decisions when developing a tissue repository is to determine what tissues and what processing

and other services will be provided to investigators. The potential services needed by investigators could be determined by surveying the tissue needs of the investigators likely to be served by the tissue repository. The design of the tissue repository, including space, equipment, personnel, and supplies will depend on the types and processing of the tissues provided to investigators.

Services provided also affect the required resources of the repository. Potential services beyond providing tissues include delivery of tissues to local investigators, culturing cells from tissues, and extracting DNA and/or RNA from tissues. Providing multiple services may distract from the primary purpose of the tissue repository and such services may be difficult to discontinue once provided, even if they subsequently impede the primary functions of the tissue repository. If services are performed, the tissue repository should be fully compensated for the efforts and resources devoted to such services.

4. Issues Affecting Tissue Repositories

4.1. Annotation of Tissue Specimens, Clinical Information, and Demographics

The key components of specimen annotation include the age, race, and sex of the patient and a diagnostic description of the specific aliquot of the specimen provided to the investigator (*see* **Subheading 5.3**). After this basic information, the extent of annotation required for a specimen will vary with the primary use of the specimen as well as the goals of the tissue repository. For example, if the primary goal of the tissue repository is to develop a tissue bank to support complex epidemiological studies of a disease process (e.g., diabetes mellitus, type II), then detailed clinical, familial, and social histories of the patient would be collected at the time of tissue collection. In contrast, if tissues are being collected and used to study the biochemistry of a wide variety of diseases and little clinical information is needed for these studies, only basic annotation may be required and the collection of detailed clinical information associated with specimens on all patients would be a waste of resources. When detailed annotation on only a portion of a specimen collection will be needed, it is more efficient to collect the information on only the patients from whom those specimens were obtained and only when clinical data are needed.

4.2. Tissue-Processing Variables

The collection and processing variables that affect the operations of a tissue repository should be identified as part of the design of the tissue repository. Many variables such as neoadjuvant therapy exposure may limit the usefulness of specific tissues and it is important that investigators using such tissues understand these limitations.

In addition, some variables will be very limiting to the usefulness of specimens.

4.2.1. Population

Tissues are usually only available from local medical facilities; thus, some expectations should be defined regarding how many specimens of various types will be needed by the tissue repository. Similarly, needs for types of tumors and other tumor characteristics may vary. For example, if the geographic area of the tissue repository has a small Hispanic population and samples of tissue are needed from Hispanics, arrangements may need to be made for obtaining tissues from medical facilities in a geographic area with a large Hispanic population. Unless tissue repositories are already in operation at such sites, development of ancillary sites for collection of tissues for research may represent a large expenditure of effort and resources to establish a typically distant relationship. It is our experience that many such relationships fail, so these relationships should be approached and developed with great care.

4.2.2. Preoperative changes

Currently, many tumors and diseases have partial therapy prior to definitive surgical therapy. For example, many patients with breast cancer (also sarcomas and prostate cancer) are treated with neoadjuvant therapy (chemotherapy or radiation) before surgical resection. Such therapy can be very effective; however, selective populations of neoplastic cells may be destroyed completely by such therapy, resulting in a residual tumor that does not represent the original disease. Similarly, metastatic lesions may be destroyed so that correct staging of the disease is no longer accurate. It is very important to identify patients who have received neoadjuvant therapy prior to the use of residual tissue in research and to ensure that all users understand the limitations of these specimens.

4.2.3. Intraoperative Changes

Healthy, uninvolved, and diseased tissues may be damaged if their vascular supplies are cut off or are compromised. Such intraoperative changes occur while the tissue is at the normal body temperature, the temperature at which catabolic enzymes are most active. Thus, when a tissue is removed operatively from the body, many physiological and biochemical changes have already occurred. These changes are referred to as being secondary to "intraoperative ischemia" or "warm ischemia." Recent advances in robotic surgery have increased the operative time of some procedures, such as the radical prostatectomy. It is important to inform investigators of such changes, in that data from current specimens of prostate cancer may not agree with similar previous data. It also is important to record a tissue timeline so that intraoperative ischemia can be estimated (e.g., the time the operation was begun and the time that tissue was removed from the patient). This information is often difficult to obtain.

4.2.4. Limitations of Available Tissues/ Resources

With the development of new methods of screening for disease, tissues involved by the disease are typically smaller and are of lower stage. For example, many early breast cancers are now identified by mammography. The frequent use of this imaging technique has reduced the size and hence the stage of most breast cancers. More and more neoplastic lesions of the breast treated surgically are in situ disease, ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS), or invasive lesions of less than 2 cm in diameter. This severely limits the amount of breast cancer available to support research in that all DCIS as well as small tumors of the breast are completely processed for histologic diagnosis and clinical prognostic studies, so that no tissues may be available to support research other than fixed, paraffin-embedded material *(25)*. Similarly, screening with prostatic-specific antigen (PSA) has reduced the volume of prostate cancer when treated by surgery. In addition, newer imaging approaches together with the use of fine needle aspirates have almost eliminated the availability of tissue samples from metastatic diseases, especially bone metastases of breast, prostate, and lung. In addition, as discussed previously, tumor tissues are limited by preoperative and intraoperative changes *(3, 16)*.

Independent of newer diagnostic approaches, access to tissues and tumors of the brain and heart as well as small cell (oat cell) carcinoma of the lung continue to be greatly restricted because these diseases are not treated primarily by surgery. In addition, access to rare tumors such as neuroendocrine–neuroectodermal tumors, subtypes of sarcomas, pediatric tumors, and rare subtypes of epithelial tumors (e.g., medullary carcinoma of the colon) will always be very limited.

4.3. Storage of Specimens

Many investigators do not have ultra-cold storage systems to house their tissue specimens. As part of the design of the tissue repository, it should be decided if the facility will store tissue specimens that have been collected for specific investigators. If so, how long will storage be provided and whether or not investigators will be charged for storage of specimens should be determined.

The optimal method for long-term (≥6 months) storage of tissue specimens to support biomedical research remains controversial. Data are available to demonstrate that tissue specimens should not be stored at temperatures warmer than −70°C for greater than 1 or 2 months and not for any period in self-defrosting freezers *(13, 15)*; however, information regarding whether or not storage in LN_2 vapor phase is better than storage at −80°C is unavailable. At least one study has reported that bias was introduced into the study when cases and controls were collected differently and were stored at −80°C for different intervals *(26, 27)*. Our unpublished data indicate that there is no difference

between storage at –80°C or in LN_2 vapor phase for at least a 10-year period.

If specimen viability is a goal (i.e., that cells or tissue can exist in culture or as xenografts), the options of specimen preparation and storage are limited. Cells can clearly be grown in culture if a sample of frozen cells has been stored in media plus 10% dimethyl sulfoxide (DMSO). In addition, because the likelihood of a successful culture from a cell sample increases with the number of cells cultured, a short-term culture to increase the number of cells would be useful to promote viability. At this point, it is necessary to point out that such a cell culture would be mixed (e.g., tumor cells plus inflammatory and stromal cells). The literature concerning primary cell cultures should be consulted before attempting the culture of cells from solid tissue, whether stored or not. If solid tissue is frozen directly, it is unlikely that viable cells can be obtained when the solid tissue is thawed because the formation of ice crystals would lyse most cells within the tissue. There are a few anecdotal reports that freezing solid tissue in media plus 10% DMSO may permit the subsequent isolation of viable cells from a thawed specimen.

4.4. Records of Collecting, Processing, and Storage

It is important to keep detailed records concerning variables of the collection, processing, and storage of tissues. Of special importance is the documentation of "times" and temperature conditions, which permit the reconstruction of the history of each specimen. Such times and conditions include the operative time when a specimen is removed from the patient, the time and method of transport (room temperature, on ice, etc.) to reach pathology and the tissue repository, the method of processing (e.g., freezing in OCT, placed in media), and the time of processing (e.g., time of freezing). For tissues prepared as paraffin blocks, the time interval to fixation, the fixative (e.g., 10% neutral buffered formalin), and the length of fixation are important variables to record. In addition, the chemical components and time in each step of the tissue processor as well as the type of tissue processor utilized might be important to record. When such variables are identified, fields for this data should be included in the repository's informatics system.

4.5. Bias in Use of Tissue Collections

Without detailed and accurate records, bias can easily be introduced into research projects and incorrect conclusions can be accepted for research. For example, consider the comparison cases of a specific disease if the cases were to be evaluated using serum that had had multiple freeze–thaw cycles, a storage time of –80°C for longer than 6 years, and collection prior to an operation; while the associated controls had only one freeze-thaw cycle, a storage time of –80°C for less than 2 years, and collection in the community using a mobile van. Mass spectrometry and other very

Table 1
Examples of potential sources of bias in tissue sets

1. Population (e.g., racial mixture)
2. Fed or fasting state
3. Diurnal variations (i.e., time of collection)
4. Stress
5. Collection container (red top vs. separator)
6. Time to processing
7. Time to freezing
8. Temperature and length of storage
9. Freeze–thaw cycles
10. Sites of sample collection

sensitive methods might identify the differences in freeze-thaw cycles, collection methods, and/or storage times, and researchers might incorrectly conclude that there were proteomic differences between the serum from patients with the specific disease and individuals who did not have the disease. Such a conclusion would be based on the bias of the two sample sets *(13, 15, 26, 27)*. A bias of this nature might not be identified until attempts were made to validate the initial experimental results. In addition, actions that introduce bias into research studies emphasize the importance of using SOPs to ensure tissues are collected, processed, and stored as uniformly as practicable. In the example case of bias, careful records might have emphasized the differences in specimens and the conclusions might have been tested before reporting on a subset of case and control samples collected, processed, and stored more uniformly. Some of the potential causes of bias are listed in **Table 1**.

5. Quality Assurance, Quality Control, and Laboratory Certification

A strong program in quality assurance is important in any aspect of biomedical research. This includes resources and infrastructure used to support research including facilities that collect, process, store, and provide tissues to support biomedical research *(12, 17, 18)*.

Quality Assurance/Management (QA) is a general approach to management activities that focuses on operational improvements in

all aspects of all activities to ensure that a procedure or product is of the defined quality required. Quality Control (QC) is the system of technical activities that measures the attributes and performance of a process, or item, against defined standards, to verify that the stated requirements are fully met. Thus, QC is only one component of an overall QA program.

5.1. Standard Operating Procedures

SOPs should be developed for all activities of a tissue repository. The SOP permits a laboratory activity to be performed uniformly, day after day. The SOP should be written in detail so, if followed, a new employee can perform the activity just as well as an experienced employee. The SOPs should be organized in a procedure manual that is readily available for use at the bench. Changes in SOPs must only be made by authorized, supervisory personnel and should be initialed and dated by the person making the change. SOPs should be reviewed yearly and revised as necessary. The new SOP should be dated as to its revision, and copies of the old SOP should be retained in the repository files to permit review of prior versions. Employees must not deviate from current SOPs. Example SOPs for the processing of blood and tissue are provided in **Subheadings 5.1.1** and **5.1.2**.

In establishing a strong QA program, personnel assigned to the QA program have the responsibility for ensuring compliance with all SOPs and regulatory requirements, and should report directly to high levels of management concerning all QA issues. These personnel should aid in the development of SOPs for specimen collection, handling, processing, storage, and shipping of specimens. When problems in these areas are identified and/or if any specimens of poor quality are identified, personnel should initiate and participate in efforts to correct these deficiencies. Personnel assigned to QA should be responsible for designing, overseeing, and evaluating audits of overall operations regarding adherence to QA requirements.

Good Manufacturing Practices (GMP) are regulatory guidelines that can be adopted by tissue repository organizations to meet the organization's operational goals. Generally, these standards should include or address requirements of ISO9001, a document produced by the International Organization for Standardization (ISO), a worldwide federation of national standards organizations. The primary purpose of this document is to provide organizations with useful internationally recognized models for operating a quality management system. ISO standards are similar to GMP, but are more detailed and are accepted internationally. Tissue facilities should utilize ISO9001 in developing and monitoring their QA/QC programs.

5.1.1. Example SOP for Blood Products

There are several major considerations when obtaining samples of blood to support biomedical research:

- Patient consent is necessary to collect blood and HIPAA authorization is required to store any associated protected health care information (PHI) in a bank to support research. Thereafter, aliquots of blood or blood products and associated information may be provided as de-identified specimens (from which all 18 HIPAA identifiers have been removed) so no HIPAA authorization is required for distribution of de-identified specimens and associated information.
- Specimens should be processed rapidly within 2–4 h of draw and maintained cold (≤4°C) but not frozen after clotting. Specimens can be supplied fresh or frozen; however, if frozen specimens are to be provided, samples should not be frozen until after serum/plasma has been separated.
- Blood should be drawn with at least a 20-gauge needle. Drawing blood with smaller needles will increase the rate of hemolysis. If hemolysis occurs in 30% of specimens, the causes of the hemolysis should be investigated. Frequently, hemolysis is due to shearing red blood cells as they are rapidly drawn through a butterfly needle – even a 20-gauge needle if a Vacutainer tube is connected directly to a butterfly. This can partially be prevented by using a Luer Lok adapter, which reduces the draw pressure slightly, or by using a syringe to draw blood from the butterfly.
- Labeling specimens correctly is critical; barcodes are recommended for labeling. The printed bar code label should identify the blood product including the type of plasma – EDTA, citrate, heparin, or other type of sample – and the tube (red top vs. separator [tiger top]) in which serum was collected.
- Records of each step in the blood draw regarding size of aliquot, time of separation, and freeze-thaw cycles are critical.
- The potential goals for sample usage should be defined clearly (e.g., analysis by proteomics) in order for the SOP of blood drawing to be adequately developed.

Multiple organizations in which we participate have developed SOPs for drawing and preparation of blood products including the Early Detection Research Network (EDRN), the Cooperative Human Tissue Network (CHTN), and the Pulmonary Hypertension Research Initiative (PHBI). We have participated in the development of the SOPs for the collection and organizational preparation by these organizations of blood products. The following is a synthesis of these SOPs.

We recommend that larger-drawing Vacutainer tubes be utilized, with the goal of drawing enough blood at one draw to prepare multiple aliquots of the blood products – serum, plasma, buffy coat, and/or whole blood – to reduce freeze–thaw cycles. The aliquots we prefer, 250 μl, have been selected as a compromise between the labor required for the aliquoting of a sample

large enough to be analyzed by multiplex immunoassay or by mass spectrometry, and being small enough to minimize waste on thawing. Some general recommendations are:

- Consent for the specific size of the blood draw must be obtained from patients (see your local IRB for limitations) and HIPAA authorization must be obtained for storing of the blood and associated patient information in a biobank/tissue repository.
- Before aliquots are distributed to investigators, specimens should be de-identified, which includes making sure that none of the 18 HIPAA identifiers are included in the medical information provided to investigators.
- If samples are to be collected prospectively, a "request form" should be developed, which not only details the investigator's needs (e.g., 250 μl of EDTA plasma from African American women and stored at −80°C or colder for <6 years with no freeze–thaw cycles), but also contains various agreements between the investigator and the tissue repository. These agreements may include a requirement for the education of all experimental personnel in biohazards, indemnification, commercial issues, and such requirements as not trying to identify the source of specimens. These can be included in a materials transfer agreement (MTA) or similar agreement.
- If drawing from an intravenous (i.v.) port, make certain that the sample is not contaminated by i.v. fluids/medications.
- The actual procedure for drawing blood is beyond the scope of this manuscript.

5.1.1.1. Serum

A red-top Vacutainer tube should be used for drawing blood that will be processed to aliquots of serum. Note that only approximately 40% of this volume will result in serum unless the patient has a cardiac/pulmonary or other disease that produces an increased hematocrit, in which case the volume of serum may be much less. Some groups recommend drawing blood for serum (red-top tube) first to minimize contamination with Vacutainer tube additives (e.g., anticoagulants) if one needle is used for drawing multiple tubes. The time of blood draw should be recorded.

(a) After drawing, allow the Vacutainer tube to stand upright for 30–45 min (record the time) at room temperature to ensure adequate coagulation. Thereafter, keep the tube cold (4°C) if there will be a long interval (>1 h) prior to processing and storage.

(b) Centrifuge the tube(s) at 1,300 × *g* for 10 min.

(c) Transfer the tube(s) to a stable tube rack.

(d) Saturate a gauze square with 70% EtOH or use an alcohol prep pad. Place the gauze over the Vacutainer tube rubber stopper and carefully remove the stopper. Do not disrupt the clot.

(e) Using a pipette, draw off 90% of the fluid at the top of the tube. This will be the serum. Record any hemolysis. If this is an adequate amount of serum to meet the needs of the resource, proceed to **step k**.

(f) If additional serum is required, place the serum initially drawn from the tube into a 15-ml conical tube. Transfer the tube to a stable tube rack.

(g) Carefully draw off remaining serum and transfer into a separate conical tube.

(h) Centrifuge the second tube at 1,300 × *g* for 10 min to remove any contaminating red blood cells or other material.

(i) Using a disposable pipette, draw off the remaining fluid (serum).

(j) Add the second serum draw to the first serum draw. Invert the tube to mix the serum.

(k) Transfer 250-µl aliquots of serum into cryovials.

(l) Screw the lids securely onto the cryovials.

(m) Freeze the cryovials containing aliquots of serum upright on dry ice or in liquid nitrogen vapor.

(n) Store at –80°C or colder.

(o) Record the times of processing and freezing, and record the storage location.

(p) If the clot is needed for DNA analysis or other analysis, it can be removed from the Vacutainer tube and frozen in a cryovial.

(q) Discard pipette and tubes with remaining red blood cells into appropriate biohazard waste containers according to your institutional requirements.

5.1.1.2. Plasma

A lavender (EDTA) tube should be used for drawing blood that will be processed to aliquots of plasma and buffy coat. Note that only approximately 40% of this volume will be plasma unless the patient has a cardiac/pulmonary or other disease that produces an increased hematocrit, in which case the volume of plasma may be much less. The time of blood draw should be documented.

(a) If not processed rapidly (e.g., within 1 h), keep the specimen cold at 4°C, but do not allow the specimen to freeze (i.e., do not use blue ice, which may be colder than 0°C).

(b) Invert the tube several times to mix the blood components.

(c) Centrifuge at 1,300 × *g* for 10 min.

(d) Transfer the tube to a stable tube rack.

(e) Saturate a gauze square with 70% EtOH (or use an alcohol prep pad). Place the gauze over the Vacutainer tube rubber

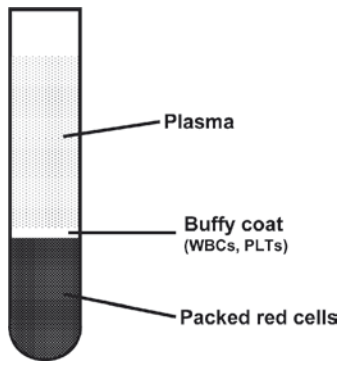

Fig. 1. Blood components separated after centrifugation.

stopper and carefully remove the stopper. Do not disturb the plasma/buffy coat layers.

(f) If the plasma accidentally becomes contaminated with white cells or red cells, transfer the plasma into a secondary centrifuge tube, and centrifuge a second time at 1,300 × *g* for 10 min to remove all potentially remaining cells.

(g) Using a pipette, draw off the fluid at the top, which will be the plasma. Be careful not to disturb the middle (tan) layer, which is the buffy coat (*see* **Fig. 1**).

(h) Transfer 250-µl aliquots of plasma into cryovials.

(i) Screw the lids securely onto the cryovials.

(j) Freeze cryovials containing aliquots of serum upright on dry ice or in liquid nitrogen vapor.

(k) Store at –80°C or colder.

(l) Record the times of processing and freezing, and record the storage location.

5.1.1.3. Buffy Coat

1. After the plasma has been removed, the buffy coat will be the next layer, which is the tan cellular material located between the plasma and red blood cells (*see* **Fig. 1**).
2. With a clean pipette, carefully draw up the buffy coat (it is likely that some red cells may be pulled up in this draw; this is acceptable).
3. There is now a choice of three pathways:
   (a) The material can be frozen neat:
      (i) Transfer the buffy coat into a cryovial.
      (ii) Freeze the cryovial upright at –80°C or colder and store at –80°C or colder.
      (iii) Record the time and date of freezing and type of storage.

(b) The material can be processed to immortalize lymphocytes; these are processed and stored at liquid nitrogen vapor phase or colder (note: the procedure for immortalization of lymphocytes is beyond the scope of this manuscript).

(c) The buffy coat can be frozen with RPMI and DMSO:

(i) Measure the amount of buffy coat and add an equal volume of RPMI together with 20% DMSO. The DMSO is 10% of the total volume.

(ii) Transfer the cocktail to a cryovial.

(iii) Freeze the cryovial upright at –80°C or colder and store at –80°C or colder.

(iv) Record the time and date of freezing, the additives, and document the type of storage.

4. Discard the pipette and tubes with remaining red blood cells into appropriate biohazard waste containers according to your institutional requirements.

5.1.1.4. Whole Blood

(a) Record the time of the blood draw.

(b) If not processed rapidly (e.g., within 1 h), keep the specimen cold at 4°C, but do not allow the specimen to freeze (i.e., do not use blue ice, which may be colder than 0°C).

(c) Whole blood is best handled fresh; freezing whole blood without 10% DMSO will cause extensive lysis.

5.1.2. Example SOP for Obtaining Solid Tissue for Research

Important considerations in the collection of solid tissue include the following:

- The clinical usefulness of the specimen must never be compromised.
- Aliquots of solid human tissues to be frozen should be relatively small (0.25 g or less) to minimize future freeze–thaw cycles and to aid in rapid freezing. The exception to this would be if samples are being collected prospectively, and samples of a specific size are requested by an investigator.
- Samples to be supplied fresh (e.g., to establish cell cultures) should be finely chopped (<0.5 mm) and placed in a standard transport media (e.g., RPMI 1640) until transferred. If it is necessary to hold the specimen for longer than 24 h (e.g., to await diagnostic review before distribution can take place), the sample should be placed in a cell culture incubator until it can be transferred. In some cases, fetal calf serum (usually 10%) and/or antibiotics/antifungals are added. Shipping to distant sites should usually be at 4°C, although some cellular components are best shipped at ambient temperature.

- A unique identification code should be assigned to each aliquot of the solid tissue. This code preferably is added via a bar code, which is linked by software to the demographic and clinical information of the donor as well as the storage site of the aliquot.
- Important times related to the specimen should be recorded and included in the information linked to the bar code, as discussed previously.

After processing, frozen tissues may be stored several ways:

- The tissue can be placed in a tissue cassette, which is then wrapped in heavy-duty aluminum foil to reduce desiccation, and labeled on the outside of the foil with the bar code.
- The tissue can be placed in a plastic cryovial, which is labeled with the bar code (note: tissue may be difficult to remove from a cryovial).
- Tissue samples may be placed in other similar closed containers that withstand –80°C or LN_2 temperatures.
- Tissue may be placed in a cryomold, covered with OCT compound, then frozen (note: OCT may interfere with some assays).
- Tissue may be placed in RNA*later* for subsequent freezing (see http://www.ambion.com/techlib/prot/bp_7020.pdf for processing of specimens in RNA*later*).

Current reports indicate that specimens should be stored at –80°C or colder. To date, data indicate that specimens degrade within 6 months at –20°C ***(13, 15)***; few differences have been shown between long-term storage at –80°C and at the freezing temperature of liquid nitrogen vapor (–186°C).

Procedure: The specific procedure used in an SOP for a tissue repository will depend on the workflow of the tissue repository, surgery, and pathology. The procedure should be organized for optimal and efficient workflow. The following is provided as an example procedure:

- On the day before surgery, check the surgical schedule and identify cases of interest that could supply needed remnant tissues.
- On the morning of surgery, distribute sterile specimen container(s) surrounded by wet ice and identified with the patient's name and hospital number to the surgical desk for distribution to the appropriate operating room.
- Immediately after removal from the patient, the operative specimens should be placed in the iced sterile specimen container. When the specimen is removed, tissue repository personnel should be informed so they can transfer the specimen to pathology. The times of initiation of surgery (anesthesia), specimen removal, and specimen transfer to pathology should be recorded, if available.

- The tissue repository personnel transport the specimen directly to pathology or the frozen section room and clearly describe to personnel in pathology the types of tissues needed from the specimen (e.g., malignant breast tissue and matching uninvolved breast from a mastectomy). Tissue not needed for diagnosis is rapidly removed from the specimen and supplied to tissue repository personnel for research.
- If space is not immediately available for processing of the tissue, it may be necessary for tissue repository personnel to transfer (on wet ice) the remnant research tissue to a tissue-processing laboratory for additional tissue retrieval, processing, and storage. Note: It may take several hours to dissect and process all research tissue from a large specimen.
- Tissue specimens may be further dissected into multiple aliquots of each specimen type (e.g., from a 1-g specimen of malignant breast and 1-g specimen of matching uninvolved breast, four 0.25-g aliquots of tumor and four 0.25-g aliquots of matching uninvolved tissue could be prepared).
- As aliquots are created, representative quality control (QC) aliquots should be taken. These could represent a 1:1 relationship with each aliquot, or a QC aliquot can be taken between two research aliquots such that the QC aliquot is representative of both research aliquots.
- Research aliquots should be processed appropriately (fresh, frozen, RNA*later*, fixed) depending on the need and identified use of the tissue or SOP of the bank.
- Each aliquot of tissue (both research and QC) should be assigned a unique code for identification. It is, however, important that the code assigned to the QC aliquot be such that the QC aliquot can be easily linked to the research aliquot(s) it represents (e.g., QC aliquot "A–B" is representative of research aliquot "A" and research aliquot "B").
- QC aliquots can be placed in fixative and processed to paraffin blocks or frozen in OCT, yielding paraffin-embedded sections or frozen sections, respectively, for microscopic examination.
- Barcode labels should be generated to allow unique identification of each aliquot of the specimen, as discussed previously in this chapter. Subsequently, additional clinical, demographic, storage location, and QC information can be added to the database.
- When specimens are to be retrieved, the bar code identifies the characterization of each aliquot as well as the storage site of each aliquot. Upon removal of a specimen from the storage location, the bar code is scanned and the disposition of the specimen (e.g., transferred to investigator X) is entered into the database for tracking.

5.2. Audits

Audits are written periodic evaluations of operating procedures and infrastructure. Tissue repository facilities should conduct regular audits such as those listed subsequently *(12, 17, 18)*. Audits may be as simple as a weekly check of freezer temperature logs or liquid nitrogen levels or may be more complex, such as a quarterly review of specimen collections. QA personnel document problems and report them to upper management, who report directly to the chief executive officer of the repository.

The QA program of a repository should describe how and when audits are conducted. For examples, specific audits and records could include the following:

- SOPS and adherence to these procedures
- Equipment maintenance and repair
- Equipment monitoring (e.g., determining the cutting thickness of microtomes)
- Training records and adherence of staff to required training (e.g., training in biohazards)

Tissue repository organizations should consider distributing an annual survey to determine the satisfaction of users/investigators who obtained tissue during the preceding calendar year. The results of the survey should be evaluated carefully especially by the QA group. Investigators reporting unsatisfactory results should be contacted and their problems discussed and corrected if practicable.

If the organization provides tissues to extramural investigators, each shipment should be monitored closely. Such monitoring can be accomplished by including a short questionnaire with each shipment that documents receipt of the shipment and any specific problems with the shipment (e.g., not enough dry ice in frozen shipments).

5.3. Quality Control of Tissues

Monitoring the quality and diagnoses of the actual tissues provided for research (i.e., quality control) is a very important component of the QA program. Tissue facilities have used various forms of QC to aid investigators with their studies in order to ensure that the tissues and associated information provided meet the needs of the investigator *(3, 6, 16)*. Many tissues, especially tumors, are heterogeneous; thus, specimens from tumors vary regarding the extent of neoplastic cells, mucin production, fibrosis (desmoplasia), inflammatory cells, and/or necrosis. Fibrosis in and adjacent to tumors may be intermixed with or mistaken for tumor and some tumors may diffusely infiltrate healthy tissues making areas of tumor difficult to identify grossly. Therefore, in general, just knowing the diagnosis of a patient from whom tissues are obtained is not adequate quality control for tissues provided for research.

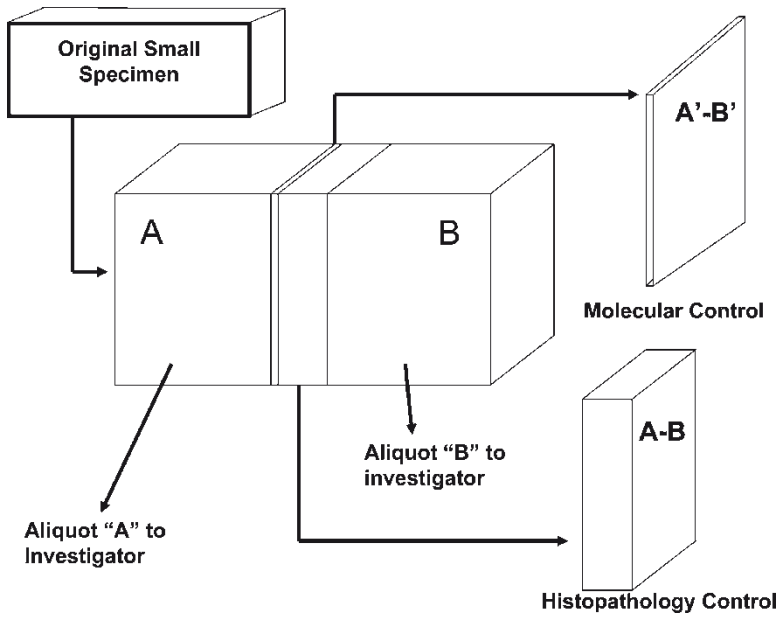

Fig. 2. Tissue specimens supplied for research.

The minimum QC for each tissue repository organization is the microscopic examination by a pathologist of an aliquot of tissue that is very representative of the specific tissue that is supplied for research. Optimally, unless the tissue is very small, a QC examination (**Fig. 2**) is made on a mirror image piece of tissue to that supplied for research. Note that in **Fig. 2**, AB is processed to a paraffin block and histopathologic examination of AB is the QC mirror image for both specimen A and specimen B. Similarly A′B′ is the molecular quality control of these two specimens.

Using this or similar methods of QC, the Cooperative Human Tissue Network (CHTN) has found that approximately 15% of tissues collected for specific research cannot be used for the specific research for which the specimens originally were collected *(3, 6, 16)*. For example, areas of tissue that appear grossly to be unaffected by disease process may be microscopically involved by disease. Conversely, specimens that appear to be diseased may include such a large component of other processes, such as scaring/fibrosis/accumulation of mucin or damage by radiation that they are unusable for specific research. Other reasons for rejection of a specimen of tissue because of the QC examination may include extensive ischemia, inflammation, or necrosis. For example, some focal areas of large tumors (e.g., large renal cell carcinomas or liver metastases of colorectal cancer) may be so necrotic that only a few recognizable tumor cells remain in the areas of tumor collected for research. Typically, in the QC examination, the

proportion (percent) of the specimen that is diseased is specified along with the percent necrosis/fibrosis of the diseased areas as well as the percent of other factors such as mucin formation. For example, a mucinous tumor may be composed of greater than 90% acellular mucin and the lack of cellular representation is likely to impede many specific forms of analysis. Another important QC index of tissues used in research is the proportion of cells within the tumor that are neoplastic. This is necessary because a tumor may be infiltrated by a large proportion of inflammatory cells. QC can also be performed on frozen sections of a specimen embedded in frozen section support medium (e.g., OCT). Note that OCT or other similar heavy alcohol-based support mediums needed to obtain frozen sections may superficially contaminate the specimen and subsequently interfere with some assays (e.g., biological assays for folate).

Figure 3 demonstrates QC via a frozen section including the minimum information needed by most investigators.

The QC examination also can be in part based on "molecular quality control" in which mRNA, DNA, and protein are extracted from small aliquots followed by molecular characterization of the molecules using various analytical methods ranging from mass spectrometry or gene arrays to examination of ribosomal bands of RNA using gels *(8)* or other systems such as the Agilent® 2100 System. Molecular quality control is performed when investigators request this level of QC and periodically to verify the quality of specimens in general provided by the facility. It may also be performed when investigators indicate that there is a specific problem with any of the specimens with which they have been

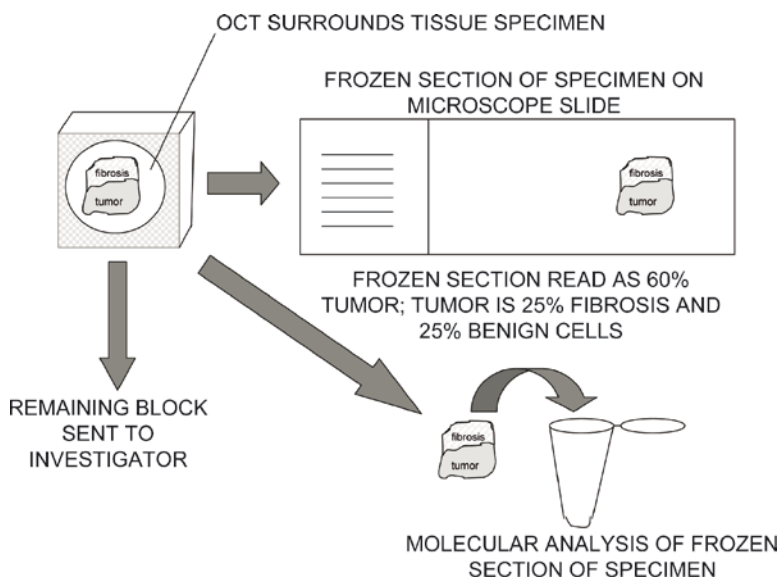

Fig. 3. Quality control using frozen sections.

supplied, if additional aliquots of those specimens are still available at the facility.

Even more extensive QC examinations of tissue are sometimes needed by investigators or specific collaborative research projects; however, as the required QC examinations become more extensive, these more extensive QC measurements have a "price" for their use; specifically, increased time and effort is put into the QC examination of the specimen by the tissue repository organization and thus there is usually an increased "cost" of the specimen to the investigator.

QC can also be tailored based on the request of individual investigators. Rarely, an investigator may request a "platinum level" of QC, which is demonstrated in **Fig. 4**. In this approach to QC, frozen sections of the whole specimen are made, followed by macrodissection to enrich the specimen in diseased cells, followed by a QC examination of the opposite side of the specimen plus additional macrodissection if necessary. In addition, aliquots of the front and back of the specimen after macrodissection would be analyzed molecularly. Thus, frozen sections from both

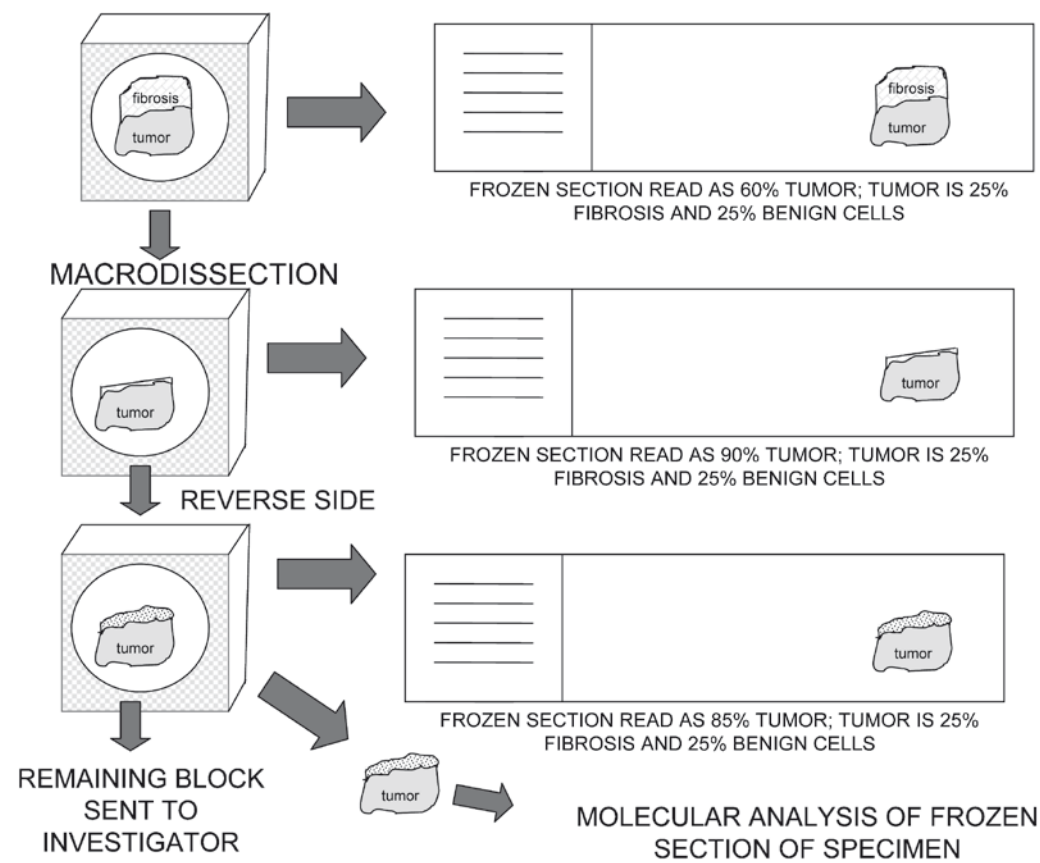

Fig. 4. Platinum level of quality control based on frozen sections, macrodissection, and molecular assays.

sides are not only used in microscopic examinations for QC, but also are used in molecular QC. Such an approach to QC increases the costs to the tissue repository of processing specimens and to the investigator. This approach potentially reduces the tissue available to support research because the QC examination begins to exhaust the specimen. If the research projects of an investigator do not require extensive QC, such an approach is not cost effective. Investigators may wish to perform molecular QC and/or microdissection (e.g., laser capture) or macrodissection at their home laboratories or organizations, hence reducing the need for extensive efforts in QC at the tissue repository. In addition, most research with today's microtechniques may not require this extent of QC.

The QC examination of tissues for research should match the research planned for the tissues and the investigator requests. For most tissue specimens and investigators, the approach shown in **Figs. 2** or **3** is adequate. If tumor enrichment or molecular analysis is required, this can be requested by the investigator or can be performed at the investigator's home institution. If mRNA is to be analyzed using long amplicons greater than 200 base pairs, a section of tissue at the request of an investigator can be examined to determine the quality of RNA using the Agilent 2100 method; obviously, if the specimen is not to be used in RNA analysis, such analysis adds unnecessary costs. Note that using short amplicons in real-time quantitative polymerase chain reaction (PCR) techniques permits the use of somewhat degraded RNA, and even RNA extracted from paraffin blocks can be used to give equivalent results to those using frozen tissues *(10, 11)*. The best and most cost effective approach for QC is to utilize a simple approach **(Fig. 2)** that can be expanded to the platinum level, but only at the request of an investigator.

5.4. Quality Assurance for the Collection of Bodily Fluids

The QA of bodily fluids primarily involves selecting the parameter of collection, processing, and storage, then incorporating these into SOPs while avoiding bias in the selection of patients and in the collection, processing, and storage of the specimens *(13, 15)*. Most studies rely on specimens of bodily fluids that are frozen within 4 h of collection. After clotting and prior to processing, the specimens can be maintained at approximately 4°C, but freezing of any blood specimen prior to processing should be avoided to prevent hemolysis. For many methods, hemolyzed specimens should be avoided, in that red blood cells may release large amounts of hemoglobin as well as proteolytic enzymes after lysis. For a discussion of other issues concerning QC of a tissue repository, see the Best Practices of the International Society for Biological and Environmental Repositories (ISBER) *(12, 17)* and National Cancer Institute (NCI) Best Practices *(18)*.

6. Regulatory and Ethical Issues in Tissue Repositories

6.1. Informaed Consent and HIPAA Authorization

Tissue repository organizations frequently obtain remnant tissues that remain unused after surgical and/or other diagnostic or therapeutic procedures have been completed; these remnant tissues would otherwise be discarded or destroyed. The local IRB determines whether or not it is necessary to obtain consent from the patients who are the source of these tissues; the local IRB may elect to waive the requirement for informed consent and HIPAA authorization from these patients *(5, 19)*.

Informed consent is not easy to obtain from such patients by tissue repository personnel because the personnel of the tissue repository have no clinical or other relationship with these patients and it has been demonstrated that personnel who do have a clinical relationship with the patients (e.g., surgical nurses or surgeons) are too busy to obtain informed consent for use of tissues in research. In addition, there is no optimal time or place to obtain the consent. Upon admission, patients may be overwhelmed and may tend to sign anything. At clinic or in the preoperative area, there may be no space or time for personnel of the tissue repository to obtain consent; also, many clinics operate simultaneously, requiring multiple personnel to be involved in the consent process. After surgery, patients may be on pain medications that may affect their ability to provide informed consent. Also complicating the ability to obtain postoperative consent is the fact that many patients may be discharged on the same day as their surgery. Thus, there is no perfect time and place to obtain informed consent. We have found that obtaining informed consent typically requires approximately 30 min per patient. Since thousands of patients per year may be sources of tissue, obtaining consent from individual patients is very expensive, usually requiring more than one full-time person.

While obtaining informed consent, written authorization of patients to use their protected health care information in research also can be obtained. Although the description of the research may often be too general to meet HIPAA requirements, obtaining HIPAA authorization from patients may support waivers of HIPAA authorization by the Privacy Officer/Board.

6.2. Cost Recovery

It is illegal to sell human tissues; however, it is legal and ethical to recover the costs associated with collection, processing, storing, and providing tissues to investigators. Funds collected in cost recovery greatly aid in the support and maintenance of a tissue repository. A primary issue is to determine the proportion of cost recovery and establish a processing cost per specimen. For some repositories, grant support may cover some of the costs; therefore, processing fees for tissues may be set to approximate the costs to that of an experimental animal.

7. Safety

The personnel of a tissue repository are exposed to many potential sources of injury including biohazards, chemical hazards, electrical and physical injuries, and fire dangers. To minimize these dangers, a tissue repository must develop a safety program. This safety program can be a component of an overall safety program of any institution with which the tissue repository is associated. Such a safety program will also provide a safety committee and safety officer to work with the tissue repository. Other articles may aid a tissue repository in establishing a safety program *(7, 14)*.

7.1. Biohazards

One of the initial decisions that a tissue repository should make is whether or not to collect tissues from patients infected with human pathogens (e.g., HIV, hepatitis B) or patients at risk of infection (e.g., i.v. drug abusers). Many tissue repositories have elected not to collect such tissues; nevertheless, any tissue repository might accidentally or unknowingly provide numerous investigators with infected tissue. It is therefore important that tissue repositories require that all personnel processing or using the tissues the repository supplies be educated in biohazards and handle all tissues with universal precautions. Those receiving the tissues also need to sign an indemnification agreement with the tissue repository, i.e., to hold the suppliers not responsible for injuries caused by the tissues or tissue products that are provided. In addition, the personnel of the tissue repository must be similarly educated in biohazards and should be provided with vaccinations for hepatitis B.

7.2. Chemical Hazards

Exposure to hazardous chemicals such as formaldehyde and xylene present potential dangers to personnel of a tissue repository. This may be via direct contact or by exposure to toxic vapors. A safety program including the correct use of safety equipment to minimize exposures to hazardous chemicals is an important component of any tissue repository. A component of this safety program is yearly education in chemical hazards and the maintenance of an inventory of all chemicals along with their material safety data sheets *(14)*.

8. Informatics

An informatics system should be developed or chosen based on the size and type of the tissue repository. In general, its use should save time for the personnel of the repository, so information should be easily added to and obtained from the database. One very useful aspect of an informatics system, even a simple one, is one that is based on the use of a bar code to identify

uniquely specimens as discussed in **Subheading 3.1** on sample identification.

As suggested, when multiple time points of tissue collection, processing, and storage are to be recorded, fields for these time points should be included in the database. All information needed to develop a "history" of each specimen should be incorporated in separate fields of the database.

8.1. Vocabulary

The approach to vocabulary of an informatics system that must frequently interact with the requests from investigators for solid tissues and bodily fluid is different from an informatics system that deals primarily with diagnostic vocabulary used by pathologists. For example, an investigator may want serum from African American patients with any breast cancer; in contrast, pathologists seldom make a diagnosis of "breast cancer," but rather pathological diagnoses usually are very specific ("ductal carcinoma, well differentiated"); race is not a component of the diagnosis, and surgical pathologists rarely deal with bodily fluids. Thus, the approach to the vocabulary of an informatics system for a tissue repository must be more flexible than that of a system relying on diagnostic vocabulary. This difference is discussed and demonstrated by Edgerton et al. *(28)*.

Databases that contain identifiable patient health care information (PHI) must meet HIPAA security requirements including installation on a secure server behind a firewall. HIPAA requirements require prevention of unauthorized access to such a database so that access is only via unique individual access codes. Usually these codes permit each type of user specific types of access. Some personnel codes permit read-only, some permit data entry and editing, and some administrative codes permit modification of the database fields. One of the major HIPAA requirements for an informatics system containing PHI is maintenance of an audit trail that records all individuals who access the database even for read-only activities.

8.2. caBIG

The caBIG program is being developed by the NCI to provide consistent informatics approaches to cover most NCI activities, especially work areas associated with NCI Comprehensive Cancer Centers. Of great importance is that databases of various comprehensive cancer centers can communicate with each other. In the area of human tissues, a program, caTissue, has been developed by caBIG as an entry informatics program for use by tissue repositories. This program is available at no cost to nonprofit users. Similarly, the program TissueQuest has been developed for large tissue repositories by the CHTN to permit electronic communication concerning investigators and their tissue requests *(28)*. TissueQuest is primarily a program to permit a large tissue repository to work with the requests of numerous investigators.

9. Future Directions of Tissue Repositories

Based on guidelines and the new Best Practices of ISBER *(12, 17)*, as well as the NCI *(18)*, detailed records will be required to develop a "history" of each human tissue stored in a tissue repository. This will require an informatics program that can incorporate such data. In addition, a process of harmonization in required activities such as collection of informed consent and HIPAA authorization is being developed. These changes are likely to be applied to all repositories funded by any governmental agency. Tissue repositories should follow new versions of ISBER as well as NCI Best Practices related to human tissue repositories. Similarly, informatics systems of tissue repositories should follow HIPAA security requirements as well as the new requirements developed by caBIG.

10. Summary

Human tissue specimens of superior quality, as well as matching clinical data are needed to support many types of biomedical research. As we have discussed, there are several issues that should be considered and addressed when attempting to meet this need through the design and establishment of a human tissue repository.

Acknowledgments

Our work is supported in part by the Cooperative Human Tissue Network (NCI #3U01CA044968), the Breast SPORE at UAB (NCI #1P50CA89019), the Pancreatic SPORE at UAB (NCI #P50CA101955), the Early Detection Research Network (NCI #5U24CA086359), and the Cardiovascular Medical Research Education Fund (TP1GR).

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