Chapter 2

Getting Started: Developing an Institutional Resource Within the Pathology Department

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When considering the location of a newly developed biospecimen repository, the pathology department is a logical and reasonable site in which to locate this facility. However, biospecimen repositories, also known as *biorepositories* or *biobanks,* have not usually been developed along centralized lines, and therefore the establishment of a new biorepository in light of several existing (satellite) biobanks may be somewhat challenging. The goal of this chapter is to explore the role of the pathology department in the management of biospecimens, discuss specific needs regarding specimen type, and explore the relationship between satellite versus central banks and ways in which to streamline this process.

The Pathology Department as a Central Processing and Tissue Management Hub

The pathology department is central to the collection, processing, storage, and distribution of high-quality patient specimens. These specimens range from solely frozen tissue to bodily fluids (blood, urine) to formalin-fixed paraffin-embedded (FFPE) tissue. Specific topics that require pathology assistance include the following:

- Diagnostic issues related to tissue, blood, or fluid collection
- Tissue allocation from a collected sample (gatekeeper function)
- Assessment of tissue and/or fluid quality
- Distribution of biobank tissue in conjunction with available material from patient diagnostic material

To ensure that tissue removed from patients receives the appropriate diagnostic attention, it has been recommended that all such tissue be examined by a pathologist. Specifically, before removal of a portion of the specimen for research purposes and/or biobanking, adequate gross pathology assessment is an essential part of diagnosis. Furthermore, essential portions of the specimen, such as tissue at the margin, tissue from the deepest portion of tumor invasion, and tissue from heterogeneous areas of tumor that require sampling, should be performed with accuracy to ensure an adequate final diagnosis.

After appropriate gross assessment has been performed, a portion of normal and/or tumor tissue (in the case of solid specimens) can be collected without compromising diagnosis. Although a maximum amount of tissue for biobanking purposes is desired, in limited cases 50 to 100 mg of tissue is often adequate to obtain useful DNA and RNA amounts for subsequent analysis. At the time of biospecimen collection, an adjacent section of tissue should be processed for frozen or FFPE histology analysis to assess necrosis and tissue content; in some instances, a tissue shave of the directly biobanked material can also be used for these purposes. The histologic findings are then catalogued within the informatics system used by the biorepository.

In general, frozen biobanked material is held for distribution until a final report on the diagnostic permanent material has been rendered. In the interim, it is the responsibility of the biobank facility to check biobanked material for histologic findings and relay these to the diagnostic pathologist. As an example, a frozen or FFPE section from the biobanked material should be evaluated not only to assure quality control parameters (eg, necrosis, percentage tumor) of the material, but should also be reviewed by a pathologist to ensure that all relevant information related to patient diagnosis (eg, depth of invasion, presence/absence of tumor) is relayed to the signing pathologist; rarely, the main diagnostic material may be present only in the biobanked material. It is recommended in these instances that frozen material be released for subsequent diagnostic analysis. On occasion, nonredundant tissue may be selected by a consented protocol. Such examples include an additional biopsy taken during early tumor analysis or a portion of biopsy material that is consented and submitted only for biobanking purposes. For accurate diagnosis in these instances, it is also recommended that a histologic section of biobanked material be prepared and reconciled with the final clinical diagnosis made by the pathologist. In limited specimens, recent work

has suggested that performing tissue imprints of the biopsy may yield useful material for subsequent analyte analysis.[1] After material has been collected and stored, it is the purview of the biobank to assess the amount of tissue available for use; aliquot this tissue and/or fluid into appropriate amounts for whole storage or DNA, RNA, and protein analysis; and monitor how much tissue remains within the biorepository. Depending on the location, additional services, such as tissue microarray construction and research immunohistochemistry, may be incorporated into the biospecimen facility.

Adequate tissue quality is essential for reproducible and meaningful research findings derived from biobanked specimens. As such, analysis of frozen or FFPE sections of biobanked tissue (or adjacent tissue) is critical and requires pathologist interpretation. In addition, because most solid tumors are composed of a complex mixture of cells and tissue, the expertise of a pathologist to assess the percentage of necrosis, percentage of tumor cells, and type of material available within a specimen (for example, the presence of normal background tissue) is needed to determine whether a particular biobanked specimen can be used directly or whether it requires further dissection, such as the use of laser capture microdissection.[2] Depending on the core functions of an individual biorepository, DNA, RNA, and protein analysis may be performed. Pathologists are in a unique position to evaluate additional material from the patient, such as paraffin-embedded biomaterials, that may better serve a research purpose and yield more representative tissue content (in contrast to the frozen material). Finally, the pathology department is primarily responsible for storage and disposal of human specimens in compliance with state and national laws. As such, locating a central biorepository within a pathology department makes both practical and legal sense.

Storage of Biospecimens by Subtype

The three general categories of biobanked specimens (which will be considered separately, below) are as follows:

- **Solid tissue or cells,** including tissue in excess needed for diagnosis and nonredundant consented tissue obtained from directed sampling
- **Blood products,** including whole blood, plasma, serum, and buffy coat prepared from blood
- **Body fluids,** including urine, saliva, buccal swabs, and so forth

Solid tissue, derived from either normal or diseased organs, may be obtained under the auspices of redundant or nonredundant tissue protocols. In the case of redundant material, tissue in excess of that needed for diagnosis can be collected under normal gross pathology protocols and stored. In contrast, tissue collected from nonredundant specimens (eg, additional biopsies obtained from a prostate during routine sampling or a portion of a bladder transurethral resection that would otherwise be entirely submitted for diagnosis) requires additional protocols. Because this latter tissue is primarily dedicated for research purposes, a separate consent process for these directed specimens are required by most institutional review boards. Furthermore, procedures to oversee the chain of custody of these tissues should also be in place. In most instances, frozen or FFPE quality control slides of the biobanked specimen would be prepared and reviewed by a pathologist. These results would contribute to the final diagnostic pathology report and should reduce concerns about "missed diagnoses." In situations where the research protocols destroy the tissue (eg, dissociations for cell line development) or utilize a large portion of the collected tissue, the clinician and pathologist should ensure that diagnostic material is still available. Optimization of protocols to prevent warm ischemia time and thus reduce degradation of molecular derivatives is essential and may include freezing tissue within the operating or procedure room in the form of small aliquots of approximately 100 mg each. Documentation of the warm ischemia time in all cases should be attempted. Table 2-1 provides some recommendations for tissue biobanking that can optimize quality.

Another common biospecimen type consists of blood and derived blood products, including plasma, serum, and buffy coat, which may be used for analysis of germline DNA. Collection of blood samples from consented patients may be performed in association with routine clinical care and can be acquired without an additional needlestick in many cases, including procuring an extra tube during clinical blood draws or collecting from an intravenous line placed during surgery. Extraction of RNA or DNA can be performed from whole blood directly, or the blood may be processed to obtain plasma, serum, and buffy coat for cryopreservation. When plasma separation is desired, use of collection tubes containing an anticoagulant (eg, EDTA, citrate, or heparin) is recommended. In contrast, collection of serum may be optimized using tubes that are coated with no additives or with a clotting activator. For specific protein-based studies, protease inhibitors can be added to avoid extensive degradation of the proteins during sample processing. The procedures outlined in Table 2-2 are

Table 2-1. Procedures for Tissue Collection and Processing

Preoperative Considerations	■ Patient consent is completed before surgery. De-identified/anonymized sample may be collected if a waiver of consent has been obtained.
Operating Room	■ Never place tissue intended for biobanking in formalin (with the exception of a quality-control section, if desired). ■ As soon as the operative procedure allows, transport the fresh surgical specimen immediately from the surgical suite to the pathology receiving room. ■ The specimen should be placed in a sterile container, and a patient identification sticker should be present. ■ Ensure that the requisition form is finalized, record the excision time, and notify the pathologist and biobanking staff.
Pathology Suite	■ Processing the tissue specimen should take place in the pathology suite. ■ The pathologist, resident, or pathologist's assistant assesses as soon as possible if there might be tissue available for biobanking. ■ Macroscopically describe the specimen according to routine protocol. ■ If there appears to be excess tissue available, the pathologist, resident, or pathologist's assistant contacts the biobanking staff. ■ Use clean utensils and work on a clean surface at all times. ■ Separate blades should be used for sectioning tumor tissue and normal tissue to avoid cross-contamination. ■ Dissect the specimen using clean instruments between dissection of normal and tumor tissue. ■ Take representative parts of the specimen for routine diagnosis as priority, and decide if there is excess material available for the tissue bank. ■ Samples selected by the pathologist are put on separate, clean surfaces, such as prelabeled dishes, for creating aliquots. ■ Collection of normal adjacent tissue from a tumor case should be performed whenever feasible.
Biobank Personnel	■ Upon receiving the tissues, biobank personnel assure that each specimen sample corresponds to its patient identifier. ■ Prepare the tissue aliquots for storage on a clean surface and use clean instruments. ■ In the ideal case, where sufficient tissue is available, a maximum of six samples (each approximately 0.5 cm^3) of every available state (if present, tumor/premalignant/normal) will be frozen. This may only be done if the diagnostic process will not be disturbed. ■ Prepare each sample for storage on a clean surface and use clean instruments. ■ Tissue for snap-freezing is placed in labeled cryovials and snap-frozen using liquid nitrogen. ■ The tissue cryovials are stored at -80°C or in liquid nitrogen. Record the location and position of cryovials. ■ Record the time of freezing. Ideally, no more than 30 minutes should elapse between time of excision and time of freezing of the tissue sample. ■ Tissue prepared for use with optimal cutting temperature (OCT) compound and paraffin blocks should be oriented with shared cut face down for OCT/paraffin block production. – OCT block production: Place a few drops OCT into mold, orient tissue, cover tissue with OCT. Position the mold at the liquid nitrogen gas–liquid interface until the OCT becomes opaque; store at -80°C until use. – Paraffin block production: Place cassettes into fixative solution pending and proceed to the tissue processor. Routine embedding and production of a hematoxylin-and-eosin–stained slide will be used for quality assurance purpose.

suggested for blood collection and processing for biobanking.

Finally, body fluids such as urine, saliva, pancreatic juice, and various effusions may be biobanked. In many instances, collection and storage of these specimens is often investigator or protocol driven, with most fluids simply aliquoted and frozen intact. For the majority of body fluids, standardized protocols and best practices have not been developed, although methods such as enrichment for cellular content and use of cyropreservatives to prevent cell lysis may be of value, depending on the study.[3,4] In general, snap-freezing samples in liquid nitrogen may be ideal for cryopreservation of the fluid product because it has been shown to yield minimal effects on assays using some fluids, such as saliva.[5] In contrast, specimens

Table 2-2. Procedures for Blood Collection and Processing

Preoperative Considerations	▪ Patient consent is completed before blood drawn. De-identified/anonymized sample may be collected if a waiver of consent has been obtained.
Operating Room	▪ Obtain blood specimen in 1-3 anticoagulant-containing tube(s) (preferably EDTA for plasma and buffy coat fraction) and serum tube(s) coated with a clotting activator, taking note of the approximate procedure time on the requisition form. Tubes may be left at room temperature and a patient identifier sticker applied to each of them. ▪ Transport the blood sample immediately to the biobank, or contact biobank personnel to collect sample. ▪ The specimen should be placed in a sterile container to which a patient identification sticker is applied. ▪ Ensure the requisition form is finalized, record the excision time, and notify the pathologist and biobanking staff.
Biobank Personnel	▪ Upon receiving the blood sample, biobank personnel assure that each specimen sample corresponds to its patient identifier. ▪ The whole blood can be processed directly for DNA or RNA according to protocols described in other chapters. ▪ Obtain a plasma-buffy coat fraction and a serum sample for cryopreservation (instructions follow, below). ▪ **Separation of plasma from the cellular fraction** 1. Centrifuge the collection tubes that contain the blood specimen at 1500-2000*g* for 15 min at room temperature. This step will separate the blood into three visible layers: – The upper layer, which consists of plasma, is generally clear and pale yellow. – The buffy coat, or leukocyte layer, is narrow and grayish white. – The bottom layer, which consists of red blood cells (RBCs), is dark red. 2. Using an appropriate disposable transfer pipette, collect plasma, divide into aliquots, and place into labeled cryovials. 3. Collect buffy coat and wash with RBC lysis buffer to remove RBC contamination and repellet; place aliquots into labeled cryovials. 4. Collect RBCs and place aliquots into cryovials. 5. Snap-freeze all cryovials in liquid nitrogen. 6. Transfer cryovials to a storage box, and place the box immediately in the -80°C freezer or in liquid nitrogen. 7. Record location and position of the cryovials. ▪ **Separation of serum from blood samples** 1. Incubate the blood in serum tubes for 1 hour at room temperature to ensure complete coagulation. 2. After incubation, centrifuge the serum tubes at 1500*g* for 15 minutes. 3. Collect the supernatant (ie, serum), and transfer aliquots directly to the labeled cryovials. 4. Snap-freeze all cryovials in liquid nitrogen. 5. Transfer the cryovials to a storage box, and place the box immediately in the -80°C freezer or in liquid nitrogen. 6. Record location and position of the cryovials.

such as urine may require additional processing, including centrifugation or filtration, for optimal results.[6-9] Although studies on nonblood fluid storage are limited, general consensus is that storage of fluids at -80°C or in liquid nitrogen may be the best option until more detailed protocols become available.

After specimens have been collected, it is essential to incorporate appropriate clinical data and metadata into the record to allow for appropriate and insightful specimen use. Metadata collected at the time of biobanking include patient demographics, specimen size and weight, frozen section information, warm ischemia time, and pertinent information regarding gross pathology. Patient clinical data is usually captured through the use of a clinical data form or, in some systems, can be attached through an electronic linkage from the hospital research information warehouse. A copy of the consent form should be available for auditing purposes and may be held by a computer-based database system. A further discussion of information technology (IT)-based issues is covered in chapter 7, *Biorepository Informatics*.

Interaction of a Centralized Biorepository With Investigator-Based Satellite Banks

Within the same institution, two different types of biorepository may potentially exist: the central, institutionally supported biorepository; and separate, smaller, individually run satellite banks. In most cases, the concept of a centralized biorepository has been relatively recently developed. To support the cost and infrastructure of such a central facility, broad-scale institutional support has been required. In contrast, many institutions have a large number of investigator-based satellite banks. Such banks have arisen out of necessity to support individual research and/or clinical trials, which are often disease centered. The coexistence or development of a central biorepository can create varying degrees of conflict with existing satellite banks. It is important to work through the processes, principles, and values of the biorepositories before embarking on the idea of a central biorepository. Each system (satellite and central) has its pros and cons. The successful, cooperative management of both systems is best accomplished by having discussions with the stakeholders of each before making the final decision to implement a central biorepository.

The concept of a central biorepository has been advocated to allow for broad-scale collection, processing, storage, and distribution of normal and disease tissue for use in basic and translational research, as well as in support of clinical trials. With institutional support, such a central biorepository could offer significant advantages to investigators and clinicians, such as detailed pathology support and analysis of tissue, standardized quality control of tissue, adequately funded storage space that allows for back-up freezers, and access for investigators to specimens from a broad array of tumor types. However, in many instances, such a centralized and robust infrastructure has been widely absent in most institutions. In these situations, investigators have developed their own directed satellite banks that have allowed for collection of necessary patient specimens and ensuring that appropriate consent has been obtained and that patients are followed up. In many instances, such satellite banks have required extensive investment by the investigator, including allocation of research funds and development of customized storage and databases that allow for information and specimen retrieval. It is therefore no surprise that owners of satellite banks may be wary of the inception of a central biorepository, thus leading to potential conflict.

There is no clearly defined way in which to readily merge existing satellite banks with a newly developed central biorepository (or even to determine if this should be undertaken). In many cases, satellite banks raise a number of variables regarding specimen collection and storage protocols, quality assessment of tissue specimens, and discrete database elements that may not be readily generalizable into a central network. Furthermore, the personal investment of research time and resources by the investigator gives rise to a sense of "ownership" over specimens that have been stored within such a satellite facility. Finally, performing a retroactive assessment of all satellite-bank–derived specimens that would include required quality elements set by a central biorepository may take up significant personnel time that can be better used for prospective specimen collection. Thus, in institutions that are developing a central biorepository, a cooperative relationship with existing satellite banks may be of value. Specifically, encouraging satellite banks to share existing tissue with interested investigators and encouraging satellite banks to utilize the central biorepository for future collections may be valuable to enhance tissue use, reduce costs, and maximize quality. Such an arrangement may allow the investigators who manage satellite banks to prospectively make use of new resources and facilities developed by a central biorepository, without losing control over specimens to which they have devoted significant financial and research time commitments.

Balancing Departmental Versus Institutional Needs for a Central Biorepository

A centralized biorepository has the potential to satisfy the needs of both the pathology department and the institution, with the caveats that adequate funding is assured for infrastructure and priorities for tissue use are established by general agreement. Methods by which to streamline agreements about specimen use include the establishment of an oversight board for the biorepository, a clear mission for the role of the central biorepository, involvement of stakeholders along disease-specific or organ-based lines, and a clear prioritization of specimen allocation and use. Such oversight and regulation have become increasingly important as the number of tissue and body fluid requests have increased to meet the needs of translational research and new drug development.

In general, there are three major uses of specimens that are stored in biorepositories. First, a primary role for the central biorepository is to facilitate clinical trials and/or therapeutics that are specifically designed to improve patient care. Such a role could potentially include tissue procurement to support ancillary studies as part of a national or multi-institutional clinical

trial. Other such efforts might include gene sequencing for specific mutations related to clinical therapeutics or participation in efforts such as the Cooperative Human Tissue Network (CHTN, www.chtn.nci.nih.gov). Such endeavors promote the institutional mission of enhancing patient care and support the role of clinicians who are actively involved in clinical trials. Remuneration from such trials may be modest and should be directed specifically to the department housing the biospecimen repository to support such efforts.

A second goal of a central biorepository is to support internal investigators (either funded or nonfunded) who will use human specimens to further research discoveries. In this way, the biorepository will also facilitate the acquisition of external funding for future research. Publications and grants that arise from such an endeavor clearly enhance the academic prestige of the institution, which may attract patients seeking high-quality clinical treatment on the cutting edge of medical care.

A third goal of biorepository tissue use is to satisfy contracts between the institution and external entities, such as industry and tissue-marketing centers, thereby perhaps providing a useful source of revenue to subsidize future research and operational costs. This third arena of tissue use may be somewhat contentious, given that some rare specimen types may be leveraged for significant financial gain to the institution rather than for internal research purposes. In such instances, oversight by disease-specific stakeholders and the regulatory body of the biospecimen repository may be necessary to establish institutional priorities for rare tissue use.

Obtaining Institutional Support for Enforcement of Pathology-Based Biorepository Policies

Ideally, support for pathology-driven best practices and biospecimen collection should be obtained from the institutional leadership. It is clear that optimal tissue collection processes should be established within the pathology department, with the assistance of the pathologist, resident, and technicians who participate in the biorepository effort. Such processes may include determining the amount of tissue that can be spared for biobanking, oversight of gross descriptions of all specimens, and review of quality-control sections performed at the time of biospecimen collection. Institutional oversight is critical in both promoting awareness of standards of practice, as well as in enforcing new policies that are established by the biorepository. Furthermore, pathologists should become actively involved not only as technical experts, but also as advisors within disease-specific advisory groups or as members of the oversight board of the biorepository.

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