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Principles of research tissue banking and specimen evaluation from the pathologist's perspective

Sarah A. McDonald
Washington University School of Medicine

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Human tissue biorepositories have an increasingly visible and important role within industrial enterprises in supporting biomedical research, including the rapidly advancing fields of proteomics, pharmacogenomics, and molecular epidemiology. Pathologists play a vital but often underrecognized role in the operation of these tissue banks. Besides interpreting studies that arise from banked samples, pathologists are needed to characterize tissues for research, to conduct quality assurance programs, to assist with resource allocation decisions, and to serve an educational role for investigators using the tissues. This article describes these key principles and illustrates examples where pathologist involvement is crucial to biorepository management. Of overarching importance, pathologists play a critical role in helping biorepository users understand the principles of specimen evaluation (histologic and structural composition of tissues, and their limitations) so as to optimize the scientific benefit of the tissues. In conclusion, greater involvement of pathologists in research tissue banking will enhance the scientific utility of biorepositories.

Background

The author’s basis of experience was formed as an anatomic/clinical pathologist and as the laboratory lead (both strategic and managerial) for Pfizer, Inc.’s global tissue repository. This repository is a collection of >12,000 human and animal tissue samples, obtained from academic and commercial sources and from a growing number of clinical trials. Tissues are stored in a variety of fixed and frozen dispositions to meet various research needs. The tissues from the bank are used to support a wide variety of discovery and development programs in the company. On a volume basis, the majority of bank use is human tissue, although preclinical species tissues (especially rat, mouse, and primate) are of increasing importance. The bank assists in the acquisition, storage, archiving, and supply of tissue through a centralized inventory and database and offers tissue sectioning, frozen and paraffin-embedded tissue blocks, histologic quality assurance, and scientific consultations on a request-driven basis to company investigators. Appropriate consent and institutional review board approval is in place for all tissues and contracts, and the use of the tissues is also governed by Pfizer’s Human Tissue Policy.

This article focuses on best practices pertinent to pathologists, especially in how they interact with and contribute to the scientific mission of biorepositories. It is important to note that these principles apply not just to pharmaceutical biobanks but, in large part, to all such banks and tissue resources supporting research for which pathologists contribute. Thus, Pfizer, Inc.’s experience could serve as a useful blueprint for academic, commercial, and other biobanks in which maintaining quality assurance for tissues is crucial. Also, the main focus of this article is on human tissue, although many of the principles would also apply to the banking and evaluation of animal tissue.

Discussion

To appreciate the roles that pathologists play in research tissue banking, it is helpful first to consider the ways in which tissues (both human and preclinical species) support pharmaceutical and other research. Key examples are given below.

• Assessment of target expression in normal and disease tissue: Through the use of immunohistochemistry (IHC), in situ hybridization (ISH), western blotting, or other procedures, the expression level of the target, that is, a protein or other biomolecule with which a drug molecule or biologic substance is intended to interact, can be assessed, and a judgment can be made about the appropriateness of that target for the intended research program. A key part of this assessment is the specificity of a target for the intended tissue or disease. It is often necessary to study target expression in a wide variety of tissues, which can be often facilitated by tissue microarray (TMA) technology (discussed later).

• Toxicity predictions: Related to the point above, tissue studies can suggest potential areas of toxicity in either...
humans or preclinical species, by showing high levels of target expression in organs or tissues different from those intended. An important subcategory is cross-reactivity assessment of therapeutic antibodies, which is typically done on frozen tissues so as to best preserve the structural integrity (and thus antigenicity) of the target protein.

- Biomarker studies: Tissue studies can support or clarify efforts to develop biomarkers for diagnostic or therapeutic assessment. Such biomarkers might be measured in the tissue samples themselves (such as through IHC), or the tissues might be useful in the assessment or development of a biomarker measured through other means, such as serum or urine.

- Field of use claims: Assessment of targets in human tissues can expand the potential applicability of a given therapeutic program, such as by highlighting the expression of a target in other disease state(s) besides the one originally considered.

- Preclinical species selection: The similarities and differences in target expression between human and preclinical species can assist the selection of the most appropriate preclinical species in which the human disease can be modeled or in which preclinical in vivo toleration studies for a drug molecule under study can be conducted.

- Clinical trials: Tissues obtained before or during a clinical trial may assist with a number of objectives, including patient stratification, prognostic assessments, and pharmacogenomics studies.

**Tissue specimen workflow**

Understanding the processes by which tissues are processed and handled is helpful as a background for a discussion of pathologist roles. All tissue originates fresh at its source. From there, tissue may be used in the fresh state, such as for the establishment of cell cultures. Alternatively, portions of tissue may be flash-frozen (for maximum long-term preservation of tissue for molecular studies, including RNA and metabolomic assessments), embedded in optimal cutting temperature compound (OCT) for the purposes of obtaining a histologic frozen section or for IHC, or formalin-fixed and embedded in paraffin (for various uses including IHC, ISH, histologic assessment using routine staining). Of overriding importance are the following principles:

- Standards for the timely collection and processing of tissue are critically important, for assuring reproducible quality of tissue and its suitability for later studies. If tissues are collected across multiple sources for given programs, then it is important to assure the consistent application of good standards across the various source sites (this will be further discussed subsequently).

- Gross examination of the fresh tissue is important in many instances, so as to assure that tissue of the right location (ie, including the disease process) and preservation is taken. For tumors, this would include sections that avoid necrotic areas. If available, a pathologist can greatly assist these determinations. Additionally, tissue must not be removed in quantities that would hinder the ability to obtain a primary diagnosis, if the tissue source is a surgical pathology laboratory. Pathologists can also guide the procurement of sections that are of the appropriate dimensions to fix or freeze in a time-efficient manner.

- Preserving tissue in several preservational dispositions (fixed, flash-frozen, OCT) is optimal, if tissue quantities and laboratory workflow permit, because it allows the most flexible set of options for subsequent lab studies.

As often as possible, the tissue repository should endeavor to review histologic sections for the samples it receives into the bank, and especially samples it is contemplating disbursing for research. These slides typically are routine hematoxylin and eosin (H&E)-stained slides. For most processes and workflow paths, this review is feasible. Sections cut from OCT or paraffin blocks, in particular, are quite facile, and information gleaned from them readily applies to additional sections cut from the same block for IHC, ISH, or other studies. One problematic area for histologic review arises when a flash-frozen, non-OCT–embedded tissue specimen arrives from an outside source (typically long term at ~80°C or colder) and is intended to be used for research, typically a digestive assay. Obtaining a histologic section from such a sample would require embedding in OCT and sectioning in a cryostat; the latter requires warming to cryostat temperature, typically ~20°C. If the research and investigator needs would render this embedding process problematic, then the sample may need to be used as is, with the performance in the assay perhaps serving as a clue to the tissue’s quality. One way around this challenge is if, at the source laboratory, the tissue adjacent to the frozen tissue sample had been previously saved for paraffin embedding. In this case, review of the paraffin tissue would provide an acceptable view of the frozen tissue’s histopathology, by virtue of geographic proximity.

Another point concerns the sections cut from paraffin blocks and placed on glass slides. Ideally, if these sections are intended for use in an assay requiring preservation of antigenicity (such as IHC), they should be used as soon as possible after sections are cut, because air oxidation of the slides may lead to deterioration of the slide’s antigenicity over time.1,2 The rate at which this occurs is variable and situation dependent and may be slowed by storing cut slides in an inert environment such as gaseous N2, or coating them in paraffin, if they cannot be used right away.2 In some instances, this principle is not fully appreciated, and unstained slides may be stored under ambient conditions for weeks or months before using them, which is not completely ideal. Pathologists can play a role in educating colleagues about the recommended prompt use of their unstained, previously cut slide sections.

**Principles of specimen evaluation**

The key principles of specimen evaluation are given below, which need to be appreciated by pathologists and others who manage research tissue repositories.

- Samples received from outside sources may not be of the type or disease state advertised or may be of poor quality, that is, necrotic. This occurs more commonly than might be believed and is usually caused by inadequate tissue preservation or handling on the source’s end, a mistaken selection of tissue samples from the parent (gross) specimen, or an error in histologic review. Pathologists, of course, by virtue of their training, are well positioned to address this challenge and encourage their investigator colleagues to submit any new or outside tissue samples for a histologic
review whenever possible. Examples commonly seen include pancreas tissue (often necrotic and/or digested by the time it is procured and stored), tumor samples (which are often necrotic or nonrepresentative of the whole lesion), and dorsal root ganglion tissue (often used in pain research, these samples may contain only peripheral nerve or soft tissue without the requisite ganglion cell bodies).

- Certain cell types within a sample, such as epithelial lining, tumor cells, or an inflammatory component, may need to be isolated specifically, rather than the whole tissue being digested in an expression assay. This will depend on the research questions being addressed. Trojan et al. provide an example with atherosclerotic plaque macrophages, where this principle was used advantageously. Pathologists play a helpful role in educating their investigator colleagues about the multicompartmental, 3-dimensional nature of tissue and how this applies to their endeavors. As a result, an investigator studying lung cancer will appreciate the sample as a complex mix of tumor cells, inflammatory cells, desmoplasia, and normal and dysplastic epithelial components, with all of these contributing DNA, RNA, and/or protein signal to assay procedures. Similarly, an investigator examining a target in ciliated respiratory mucosa, in situ disease, and invasive colon carcinoma all in the same patient and organ may be the primary objective. Comparative studies of tumor and premalignant tissue from the same patient might be removed or more optimal tissue sections possible association or effect between that stated history and the tissue was made available and should consider any research purposes. In such settings, pathologists and others should ask questions about the original procedure and why the tissue was made available and should consider any possible association or effect between that stated history and the desired “normal” tissue or organ type.

**TMA blocks**

An important feature of the industrial tissue biorepository is TMA technology. By virtue of their inclusion of dozens or hundreds of separate tissue core samples in 1 block, TMA blocks provide rapid high-throughput screening for studies involving protein, RNA, or DNA targets, with improved preservation of tissue resources. A traditional TMA is a paraffin block, though frozen TMAs can be built with OCT and, though more labor-intensive in their construction, may be helpful in addressing questions involving labile markers (eg, messenger RNA and phosphorylated proteins), which are liable to degrade during paraffin embedding. Typically, TMAs are adaptable to any assay that is routinely done on tissue sections from regular paraffin or OCT blocks. Multi-species and multiorgan TMAs may further enhance the output of information, especially for comparative judgments involving target expression across various species and organ types. Importantly, TMAs also provide a high-throughput substrate of specimens for the analysis of gene expression and cell signaling pathways that are involved in carcinogenesis.7

Comprehensive overviews of TMA construction processes are available. The key steps are the selection of “parent” blocks, the coring of these blocks to produce slender tissue specimens 1–3 mm diameter, and the inclusion of dozens to hundreds of these cores in the new TMA block, along with clinical/demographic annotation, and an array map (typically a grid) guiding the user to which specimen is in each core.

Pathologists play a critical role in the construction, evaluation, and use of TMAs:

- Selecting appropriate donor or “parent” blocks for use in TMA construction, that is, the blocks containing adequate quantities of the desired tissue or disease type. It is also necessary to select an appropriate region of the blocks from which cores can be obtained.
- Configuring TMA parameters appropriately. For example, too many cores on a slide will yield smaller core areas. This raises the risk that “edge artifacts” from IHC staining...
(whereby reagent staining is artifically enhanced along tissue edges) will obscure useful information.

- Reviewing a histologic slide for TMA blocks, for quality assurance purposes—making sure that appropriate tissues are represented in cores, documenting if they are not, and communicating that information to the user group.

Operating procedures for specimen evaluation and disbursal

Based on the foregoing principles, the following additional recommendations are advised for practices within a research tissue bank in an industrial setting (these would also apply to a large extent in other settings also):

- Communication between pathologist and repository user is absolutely essential. Such communication could include any of the foregoing principles, including tissue quality, tissue review findings, and appropriateness of a specimen for the intended research purpose. Because resource allocation decisions may be necessary when competing needs require the use of scarce or valuable tissues, communication with users is critical to establish the priorities and potential benefits of each proposed use.

- The anatomic makeup of the tissue must be considered against the project goals and methods, with the overriding concern being the following question: what cells or components of the tissue are key to the study or the choice of controls? The answer will dictate not only the choice of specimen, but also perhaps the procedure used on the tissue; for example, after considering the tissue’s histology, the pathologist and investigator may decide it is necessary to enrich for the desired cell type by performing microdissection or laser-capture microscopy.

- It is often helpful to supply H&E sections of the tissue directly to the investigator, with relevant areas of disease marked, after pathologist review. For oncology specimens, this may include areas of tumor, premalignant changes, and benign normal tissue, along with information about tumor cellularity and percentage of necrosis. Relevant information can be similarly captured in other disease settings also, for example, areas of inflammation in ulcerative colitis, or regions of stable and unstable plaque in atherosclerosis.

Principles of specimen collection and processing

Specific, reproducible criteria for tissue collection are quite important for quality assurance purposes, and these should be standardized as much as possible across the multiple collection sites that the tissue repository uses as its sources. Clear, practical procedures and recommendations are needed, and good communication is essential. Pathologists managing tissue repositories, and also the bank’s users, should understand collection practices and how they might impact their studies and the quality of received tissue. Pathologists should of course also be familiar with biomarkers for specimen quality (eg, RNA integrity number) and be ready to use that information in specimen selections and in correlating with histologic findings and/or assay results.

Good specimen handling and preservation practices are important for their influence on tissue quality in biorepository management, just as they are in other laboratory settings such as surgical pathology. Such practices have been thoroughly described. Numerous lines of evidence support the influence of collection practices on the quality of results derived from tissues. There is a recent trend within some biobanking endeavors to attempt to collect very precise acquisition information, such as the exact times the specimen spends in various parts of the acquisition/processing pathway. The extent to which this is feasible will of course vary depending on the individual laboratory. However, as very basic minimums, the following recommendations are advised: Surgical samples should be processed (either formalin-fixed or frozen) as soon as possible after removal from the patient, but no later than half an hour. Autopsy specimens should preferably be processed within 4 h of postmortem time, and no later than 8 h. Specimens in formalin should be processed within 24 h to avoid overfixation (which could compromise the antigenicity of proteins). Biorepositories, especially those in close proximity to surgical pathology services, may consider coordinating their practices with a tracking sheet or documentation system, which specifies the time of procurement in the operating room and the arrival time in the pathology laboratory or other location where the specimen is fixed and/or frozen. Such a tracking system, especially if implemented in “real time” (ie, specimen transport as quickly as possible), may increase the yield of research tissue from large surgical specimens, which, absent the need for frozen sections, might not arrive in the pathology laboratory quickly enough to be useful for biobanking. Such a collection system obviously requires good chain-of-custody measures and a good cooperation and communication between the pathology laboratory and surgical areas. Biorepositories that are distant from the sites of collection (this includes most if not all pharmaceutical human tissue banks) will need to emphasize the importance of good collection practices to the sites, perhaps implementing a tracking system similar to that described above, which should yield transparent quality control data related to timely collection.

When reviewing samples, pathologists should be alert for preservational problems reflected in the histology and be prepared to alert the processing laboratory and make changes in the process flow based on such findings. Although some types of human tissues will be primarily available in quantity through autopsy procurement (eg, brain and cardiac tissue), pathologists and repository users should keep in mind the limitations of such tissues for work with sensitive macromolecules, because postmortem times are rarely <2–4 h. Pathologists should also consider the availability of specialized fixatives (eg, Carnoy’s, methacarn, acetone, and HOPE fixative) for specific research objectives. The reader is referred to formal best practice documents published by well-known biobanking organizations for additional details on quality assurance and specimen storage and transport, as well as other topics whose in-depth discussion is beyond the scope of this article, such as informed consent, informatics systems, and biosafety practices.

Still another area of growing recognition is the presence of preacquisition variables, such as patient medications, blood and fluid administration, anesthesia, blood pressure variations, and clamp time, which likely have some influence on gene expression and proteomic profiles in some cases and thus potentially on research findings. It is not likely that biorepositories will be able to control for these factors or
even document them accurately. However, this is an understudied area that will probably elicit more attention in the future and so should be followed by pathologists and others involved in biorepository management.

Conclusion
Research tissue banking is important in promoting a variety of scientific objectives, including discovery and development programs in the pharmaceutical industry. Full use of this resource requires good histopathological science and close cooperation and good communication with investigators. Pathologists are well positioned to provide scientific leadership, especially with respect to specimen quality and evaluation, and thereby maximize the scientific benefit of tissue repositories.

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Address correspondence to:
Dr. Sandra A. McDonald
Department of Pathology and Immunology
Washington University School of Medicine
St. Louis, MO 63110
E-mail: smcdonald@path.wustl.edu
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