

Biospecimens and Biorepositories for the Community Pathologist

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● Pathologists have long served as custodians of human biospecimens collected for diagnostic purposes. Rapid advancements in diagnostic technologies require that pathologists change their practices to optimize patient care. The proper handling of biospecimens creates opportunities for pathologists to improve their diagnoses while assessing prognosis and treatment. In addition, the growing need for high-quality biorepositories represents an opportunity for community pathologists to strengthen their role within the health care team, ensuring that clinical care is not compromised while facilitating research. This article provides a resource to community pathologists learning how to create high-quality biorepositories and participating in emerging opportunities in the biorepository field. While a variety of topics are covered to provide breadth of information, the intent is to facilitate a level of understanding that permits community pathologists to make more informed choices in identifying how best their skills and practice may be augmented to address developments in this field.

(*Arch Pathol Lab Med.* 2012;136:668–678; doi: 10.5858/arpa.2011-0274-SO)

BACKGROUND AND CHALLENGES FOR THE COMMUNITY PATHOLOGIST

Drivers of this transformation relate to the concept of “personalized medicine,” namely, the idea of tailoring medical management to a patient’s genetic background or specific phenotype upon presentation. While this concept is not new to the pathologist, the practice of incorporating significant amounts of genetic and molecular information into an individual patient’s evaluation is relatively new and rapidly evolving. The genetic information may include sequences from the patient’s germline DNA, expressed transcripts, epigenetic factors, cancer cell mutations, or

sequences of infectious agents present in the patient. Examples that have been widely used include identification of specific cancer mutations in selecting appropriate therapy (refer to Table 1), use of prophylactic anticoagulant therapy in pregnant women who harbor the factor V Leiden mutation, and use of human immunodeficiency virus genotyping to drive selection of highly active antiretroviral treatment.

For editorial comment, see p 590.

As the cost to sequence an individual’s genome continues to drop, it will eventually be as economical to sequence a patient’s entire genome as it will be to perform targeted assays to detect single-nucleotide polymorphisms and other sequence variations. There are numerous challenges that face the community pathologist who wishes to proactively prepare for upcoming changes in the health care environment. This article will attempt to enumerate challenges, recognizing that solutions to some of these challenges are beyond the scope of a single pathologist or small practice to implement. It is important to keep in mind that even single pathologists at small community hospitals have been successfully managing paraffin biorepositories for years in the best interests of patient care. Small adjustments and minor augmentations may be enough to initiate a process that ensures production of high-quality biospecimens, moving into an era of personalized medicine. Challenges can be grouped into several categories.

Patient Protection

- Adherence to legal and ethical requirements
- Institutional review board (IRB) process compliance
- Patient informed consent process
- Health Insurance Portability and Accountability Act (HIPAA) compliance
- Deidentification or limited data sets of patient-identifying data as required by various protocols

Quality of Tissue Samples

- Development of standardized collection protocols and integration of a quality management program
- Obtaining an accurate time from surgery to collection/processing
- Processing and formatting samples for storage
- Pathology validation/verification
- Histologic and molecular integrity assurance

Accepted for publication January 11, 2012.

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The authors have no relevant financial interest in the products or companies described in this article.

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Table 1. Disease, Gene Targets, and Associated Therapy

Disease	Target	Therapy	Source, y
Acute myelogenous leukemia	t15;17	ATRA (Roche, Basel, Switzerland)	Petrie et al, ¹⁴ 2009
Non-small cell lung cancer	EGFR	Tarceva (OSI Pharmaceuticals, LLC, Farmingdale, New York)	Roberts et al, ¹⁵ 2010
Breast cancer	HER2	Trastuzumab	Guarneri et al, ¹⁶ 2010
HIV infection	RT, protease	HAART	Hart et al, ¹⁷ 2010
Chronic myelogenous leukemia	BCR/ABL	Gleevec (Novartis, Basel, Switzerland)	Breccia et al, ¹⁸ 2011
Lung adenocarcinoma subset	EML4-ALK	Crizotinib	Bronte et al, ¹⁹ 2010
Metastatic malignant melanoma	BRAF-V600E	Vemurafenib, ipilimumab	Márquez-Rodas et al, ²⁰ 2011
Gastric adenocarcinoma	HER2	Trastuzumab	Croxtall & McKeage, ²¹ 2010

Abbreviations: ATRA, all-trans retinoic acid; EGFR, epidermal growth factor receptor; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; RT, reverse transcriptase.

Quality of Clinical Information

- Data capture effort
- Determination/standardization of data elements to capture, including the ability to capture and retrieve discrete data elements
- Prospective data collection into the future (eg, follow-up prostate-specific antigen on a prostate cancer biospecimen)
- Utilization of standards for data coding and classification (eg, World Health Organization classifications, American Joint Committee on Cancer staging, College of American Pathologists [CAP] cancer protocols, Systematized Nomenclature of Medicine–Clinical Terms coding)

Information Technology/Bioinformatics

- Sample tracking, processing, and storage management
- Security of protected health information and the link to samples
- Accessibility of data to staff (eg, via the Web, maintaining security)
- Multisite deployment capabilities (eg, multiple hospital collection coordination)
- Browsing of deidentified collected samples by the target audience

Logistics

- Standardized equipment and supplies
- Bar coding (cryogenic label stock, narrow label printing, etc)
- Inventory management
- Vapor-phase liquid nitrogen and -80°C storage
- Sample distribution, shipping
- Training of personnel

BIOREPOSITORIES VERSUS BIOSPECIMENS

In this article, *biospecimens* are defined as physical specimens derived from human subjects. These include tissue, blood, body fluids, cells, urine, infectious disease specimens, and derived products such as, but not limited to, microscopic glass slides, paraffin blocks, DNA, RNA, proteins, and metabolites. *Quality biospecimens* refer explicitly to those samples that have been created and annotated by using standard protocols within a controlled environment and maintained as such.

Biorepositories are defined as the infrastructure within which biospecimens are identified, collected, stored, and distributed. A *quality biorepository* adheres to standard operating protocols and published best practices for annotating, collecting, processing, storing, retrieving,

and disseminating biospecimens. Included are collecting and managing clinical data, quality assurance and quality control, biosafety, inventory management (eg, freezers, racks, boxes), and ethical and legal practices. High-quality biorepository practices are required to optimize the collection, processing, and annotation of samples for diagnostic and/or research use, while maintaining a financially viable biobanking operation. Such practices promote high-quality testing and analysis while reducing the presence of confounding factors in diagnostic testing that may occur owing to variability in collection and processing methods.

Biorepositories traditionally have been established to fulfill the needs of either clinical care or research. However, the line between clinical care and research has been blurring for years. With the recognition that high-quality biospecimens and biorepositories are critical for both enterprises as we enter into an era of personalized medicine, it is imperative that community pathologists who currently serve as custodians of clinical repositories recognize emerging needs. Specific use cases for tissue, blood, research, and microbiologic samples are included at the end of this article. While some of these use cases may not be practically implemented in all hospital settings, community pathologists are challenged to explore and adapt those that may apply to their local health care system.

THE IMPORTANCE OF QUALITY BIOSPECIMENS

Biospecimens factor prominently in the delivery of personalized medicine. The quality of patient biospecimens, whether fluid or tissue in origin, significantly impacts the quality of test results that may be obtained. A variety of techniques are currently used to evaluate predictive, diagnostic, prognostic, pharmacogenomic, and toxicogenomic markers, including immunohistochemistry; fluorescence in situ hybridization; direct methods of DNA, RNA, and protein analysis; and image analysis. Such testing not only helps to diagnose and classify disease processes but also serves to predict responses to therapy, select effective therapy, titer drug dosages, identify underlying risks for adverse events, and predict outcomes. What was previously considered esoteric testing has become mainstream and mandatory for the routine care of patients, whether for treatment of cancer or other conditions. Furthermore, such analyses no longer fall into the realm of conditions largely diagnosed and managed in tertiary care medical centers. Rather, such testing is increasingly used in community practices. Results obtained in community, academic, reference laboratory, and

commercial/industry settings will be directly related to the uniformity and quality of biospecimens processed and/or archived by the community pathologist.

Access to appropriately annotated, collected, and stored human biospecimens is also fundamental to the successful development of molecular tests for diagnostic, prognostic, pharmacogenomic, and toxicogenomic evaluation as well as risk stratification. The ability to procure both common and rare specimens from varied populations and body sites is also critical to providing materials that represent the spectrum of human conditions. These materials provide an essential resource that benefit patients directly in the delivery of health care, as well as in the development of multi-institutional clinical trials to develop the next generation of therapeutics and diagnostics.

FACTORS AFFECTING BIOSPECIMEN QUALITY

Factors that affect biospecimen quality span multiple areas, including the following:

- Collection
- Processing
- Testing/analysis
- Storage
- Annotation
- Capture of standardized, coded, discrete data elements
- Distribution

Development of standards to control these processes helps minimize the presence of confounding variables and increases sample utility for various analyses. Community pathologists must identify which of these variables can reasonably and practically be controlled for and/or monitored in their own local institutions. Further, the pathologist should guide placement of these data into an appropriate location so that they can be adequately used. Uncharted sections of the pathology report that are not viewable to other clinical staff, or separate information systems, may provide suitable locations. The following list covers some of the most relevant factors that pathologists should consider.

- *Collection and Annotation:* Collection processes for clinical use and consented research studies should document appropriately signed forms for clinical and/or research purposes (eg, informed consent), as well as any special religious or cultural requests for biospecimen disposal. Further resources addressing ethical, legal, and social issues are referenced at the end of this article. Annotation of tissue samples should include disease diagnosis, tissue of origin, tissue abnormalities, tumor type, tissue blood supply and anoxic status, surgical clamp time, vital statistics during surgery, administration of blood products and other fluids, administered drugs, selection of tissue or fluid samples for banking, chilling/heating/drying of tissue during handling (including cold ischemia time), total time in formalin, and attaching the annotated data to the biospecimen. Fluid samples should, at a minimum, follow annotation as required for testing in a Clinical Laboratory Improvement Amendments of 1988 (CLIA)-certified laboratory. Annotated data sets may be bolstered as required for specific research use.
- *Processing and Preservation:* Factors to be captured regarding processing and preservation of tissue samples include chilling, heating, or drying of tissue during

handling; size of tissue pieces; amount and location of tumor, necrosis, inflammation, fat, fibrosis in tissue; tumor content and percentage of viable tumor nuclei; liquid collection media (eg, none, saline, Roswell Park Memorial Institute [RPMI], Buffalo, New York] medium [Sigma Aldrich Co, LLC, St Louis, Missouri]); use of gauze wrapping; additives (eg, antibiotics, amphotericin B) and embedding compounds (eg, paraffin, optimal cutting temperature medium, aluminum wrapping); variations in fixation (eg, temperature, buffer, and pH of formalin, proprietary additives in formalin, age of paraformaldehyde, start time and duration of fixation) and freezing protocols; use of isopentane; time to freezing; size of tissue frozen; variations in personnel; and incorporation of the cold ischemia time, type of formalin, and total time in formalin into the pathology report.

- *Storage and Tracking:* Variables in storage conditions include the type of storage, storage temperature and temperature variations, duration of storage, storage procedures and vessels allowing different levels of dehydration, desiccation and temperature gradients, laboratory power failures, appropriate monitoring, documentation and retrieval of these activities, catastrophe planning, bar coding and maintenance of chain of custody for all specimens, and exit strategy if the biorepository cannot be sustained.
- *Distribution and Usage:* Factors to consider include thawing techniques, number of freeze/thaw cycles, transportation conditions, involved personnel, procedures for returning remaining specimens to storage or re-aliquoting of existing specimens, variations between and within analysis techniques, and mechanism for recalling biospecimens whose use has been revoked by the donor.
- *Documentation:* Proper documentation includes access to standard operating protocols with management of documents and data metrics within a laboratory information system, electronic medical record, or biorepository database.

Many of the critical steps to be followed in establishing and running a biorepository fall within existing procedures in today's CLIA-certified laboratories or can be implemented with modifications to existing procedures in use in CLIA-certified laboratories. Use cases related to specific types of biorepositories are summarized at the end of this article.

ESTABLISHING A BIOREPOSITORY

Establishing a high-quality biorepository requires dedicated up-front effort for strategic planning and implementation. We strongly encourage staff to understand the business needs and end users of samples, for diagnostic as well as research purposes. Components to be considered in establishing a biorepository include the following:

- Business model and plan regarding how the repository will support clinical and/or research operations. The plan should include development of a fee schedule and methods through which end users will be billed for services provided, as well as a plan for marketing the biorepository;
- Physical space for operations and equipment, including planned expansion should the biorepository grow significantly over time;

- Staffing by trained personnel to perform operations and work with clients and other end users;
- Equipment for processing and storage, including monitored freezers, computers, tissue processing equipment, and other items;
- Reagents and supplies necessary for quality collection, storage, and retrieval;
- Information technology (IT) infrastructure for sample tracking and management, annotation and other methods of data collection, from the clinical record, or as required;
- Standard operating procedures that incorporate a quality management program for quality assurance and ongoing quality improvement.

In many circumstances, the infrastructure already used by a pathology laboratory for clinical care may be leveraged to establish a biorepository. Explicit documentation for billing of services and resource use that spans both clinical care and research is critical.

The operation of a biorepository separate from a specific IRB-approved research protocol should be approved by the IRB, including appropriate patient informed consent forms under which the biorepository operates. Biorepositories also may define procedures under which anonymous or deidentified samples will be collected and released, particularly when dealing with fluid samples and derivatives. These procedures should be reviewed and judged exempt from the need for IRB approval by the IRB and included as part of the repository's umbrella protocol providing oversight for its operations. Users of materials collected by the biorepository also may require their own IRB protocols for access and use of samples and data maintained by the biorepository. Institutional review boards generally require that protocols be renewed annually, with an update of operational data from the prior year of implementation to include demographics of patients enrolled, consents versus refusals, and adverse events, among other data elements. Information technology to support management of data related to repository operations is discussed below.

Commercial organizations exist that can assist in establishing a biorepository and/or in recuperating costs associated with the endeavor. Biospecimens that might otherwise be discarded can be licensed and used to subsidize collection and storage of retained/archived biospecimens. Many commercial entities can provide assistance with brokering tissue access in exchange for licensing fees.

CONSENTED REPOSITORIES AND INSTITUTIONAL REVIEW BOARD PROTOCOLS

Informed consent is generally considered the beginning of a series of biobanking "events," with the ultimate goal of collection of high-quality biospecimens. The selection of human subjects for participation and informed consent is a prerequisite to this step. A typical community practice may choose to focus on a readily accessible patient population, specimen type, or disease type. A number of factors come into play in generating "specimen desirability guidelines" that consent nurses may use to prioritize which patients should be approached. Factors such as recuperation of costs, demand by the scientific community, resource limitations, and logistic constraints may all play a role in how such guidelines are formulated and

used. Consent forms should document specifically what the risks and benefits are to the patient and what will and will not be permitted in relation to any collected biospecimen. Conflicts of interest between immediate patient benefit and future benefit to others for care, research, or profit must be judiciously managed. Consent content can vary from focusing on exclusions to disclosures to waivers. Complete consent may be obtained for identified tissue with allowances for treatment and outcome information with direct contact of the patient as needed. An IRB can assist in helping to craft an appropriate informed consent for a given goal.

For research biospecimen collections, establishment of an institution-wide IRB protocol for biospecimen collection is useful because the biorepository then can be used by researchers without the need for additional IRB submissions, provided that patient privacy and confidentiality are protected.^{1,2} Material transfer agreements can be established with entities outside an institution for licensing tissues for various uses, provided that this is disclosed in the consent form.

BIOSPECIMEN COLLECTION

Biospecimens must be collected in a manner that does not compromise patient care. If a biospecimen must be evaluated for clinical care as well as research, a pathologist should confirm appropriate sampling for both needs, with clinical patient care taking priority in all cases. Policies must be established to ensure that patient care is not compromised. For example, many institutions will institute an "embargo period" during which tissue collected for research may not be used. This provides ample time for the execution of the clinical care process related to a tissue collection. If the tissue collected for research is required in the clinical care of the patient, the embargo period ensures that it remains available and can be transferred to the clinical care team for processing as needed. Biospecimens may be collected for storage at an institution or they may be immediately released to an outside facility for storage and/or further processing. This latter type of collection generally requires use of specific collection protocols or kits provided by the outside facility. Samples may need to be frozen, but in many circumstances they can be placed fresh into a storage medium, packaged, and transported via express shipping.

In the community hospital, most remnant tissue from diagnostic or therapeutic resections is available as 10% phosphate-buffered neutral formalin-fixed, paraffin-embedded (FFPE) tissue. Most special studies (eg, immunohistochemistry, *in situ* hybridization, fluorescence *in situ* hybridization, chemiluminescent *in situ* hybridization, ploidy by flow cytometry) performed on fixed and processed tissues have been optimized for use in FFPE tissues. This is partly because aldehyde-based fixatives, such as formalin, are excellent for preserving proteins, the targets of immunohistochemistry stains.

However, formalin fixation may be less than optimal or inadequate for some analytes. Some nucleic acids are better preserved in alcoholic fixatives, such as Carnoy (American MasterTech, Lodi, California), UMFIX (Sakura Finetek USA, Inc, Torrance, California), or Hepes-glutamic acid buffer-mediated organic solvent protection effect (HOPE) fixative (DCS Innovative Diagnostik-Systeme Poppenbutteler Chaussee, Hamburg, Germany), while many messenger RNAs are degraded. Use of alcohol

fixatives for detection of nucleic acid sequences should thus be validated against optimally fixed FFPE tissue for each analyte that will be used for patient care.³⁻⁵ Some additional fixation artifacts to consider are listed below.

- Lipids are lost in traditional FFPE processing and require either frozen tissue or fixation in glutaraldehyde or osmium tetroxide for analysis.
- Glycogen is best preserved in alcohol-based fixatives.
- Detection of active enzymes and mucopolysaccharides is best performed in frozen tissue.
- Biogenic amines are well preserved by picrates such as Bouin (Sigma-Aldrich) but are also well preserved by formalin.
- Microwave radiation may be used alone or in combination with chemical fixatives or freezing to decrease some fixation artifacts.
- Polymerase chain reaction studies may be performed on FFPE or alcohol-fixed tissues, but molecular analyses requiring longer fragments of DNA, and many RNA targets, require fresh or frozen tissue for analysis.
- Some fixatives, such as Bouin and B5 (American MasterTech), prevent molecular analysis of the tissue.
- RNAlater (Ambion, Inc, Foster City, California) solution eliminates the need to immediately process or freeze samples. A fresh sample can be trimmed down to less than 5 mm maximum dimensions, placed in this solution, and stored at standard refrigeration temperatures (4°C) for analysis of RNA and DNA (not protein) several days later (extended periods optimally require storage at -20°C or below).⁶

Biospecimens from clinical laboratories, such as fluids and microbial isolates, frequently require automated or semiautomated methods to identify materials of interest before they are discarded, given the sheer volume of biospecimens processed in most laboratories. Their use also must take into account effects of standard laboratory storage conditions on utility of materials for research analyses. For instance, DNA and antibodies remain stable under standard storage conditions in clinical laboratories, while RNA and labile proteins would be adversely affected.

The community pathologist must balance the additional expense and effort of freezing or fixing tissues in nonformalin fixatives with the likelihood of materials being needed either for research or for clinical purposes (ie, tests for clinical diagnosis may be requested on archived tissue).⁷⁻⁹

BIOSPECIMEN TRACKING AND STORAGE

Maintenance of full audit trails for all events to which biospecimens are exposed remains a requirement for a biorepository. Such granular tracking and documentation is often referred to as a “chain of custody” to indicate that the order and details of such events are maintained throughout the life cycle of a biospecimen. That life cycle may begin in advance of the actual banking event (eg, the surgical clamping time). Environmental conditions that may introduce preanalytic variables into downstream analyses are particularly important to document (eg, freezer failure resulting in a partial thaw cycle). Biospecimens must be assigned a unique identification code to initiate the audit trail. Identification codes may be assigned manually by using an arbitrary yet systematic

process, precoded containers, or with biobanking software that uses an internal sequence or “wheel” to generate unique identifiers for all samples, both primary and derivative. The codes are then physically affixed to the biospecimen container, generally with a bar-coded label. These coded biospecimens may then be electronically linked to patient data, including encounters and consents. Aliquots derived from a particular biospecimen also may be electronically linked to the parent sample. Throughout the biobanking process, the location, condition, and aliquots of a primary sample should be electronically tracked and made accessible to biobanking staff.

Biospecimen storage has been increasingly standardized. Biospecimens are generally stored in racks or boxes, depending on the physical structure of the associated containers. Frozen materials will be placed into freezers in a specific location dictated by the specimen tracking information system. Standard storage containers for paraffin blocks and slides are common within pathology laboratories. Environmental conditions should be monitored and logged with a 24/7 alarm system to alert the biorepository staff if parameters fall outside acceptable ranges, such that corrective action can be taken. A backup procedure should be developed. Processed samples are frequently arrayed in microtiter-sized containers (DNA, RNA, or protein extracts). Tissue cores from paraffin blocks may be embedded in tissue microarrays to allow analysis of multiple samples maintained within a single block.

DATA ABSTRACTION, DEIDENTIFICATION, AND ANNOTATION

Data linked to biospecimens may be simple, focusing on biospecimen metadata associated with collection, processing, storage, and release, and may encompass detailed clinical annotations relevant to the purpose for which a biospecimen has been collected. In instances for which the ultimate purpose for a biospecimen is varied or unknown, the community biorepository may rely on standards for collection of data elements from government efforts or from standard-setting organizations.^{10,11} Alternatively, a simple, secure link to the electronic medical record may provide a viable starting point for many community biorepositories.

Annotating biospecimens with data abstracted from the clinical record can readily increase the utility of the biospecimens. If broad use of biospecimens and data are not explicitly detailed in the protocol under which the biospecimens were collected, then future options for use of biospecimens by other biorepository protocols may be limited. A direct link to the electronic medical record, with automated deidentification to protect patient privacy within the guidelines of HIPAA and other federal legislation, may seem out of reach, but more recent software systems have emerged to meet such requirements. The caTissue Suite software, freely available from the National Cancer Institute (Bethesda, Maryland), includes integration with a commercially available, automated deidentification program that requires a license fee if used.¹² The i2b2 software package (Informatics for Integrating Biology and the Bedside; National Centers for Biomedical Computing¹³) also provides open-source tools to establish deidentified as well as encrypted datasets that can be linked to 1 or more biospecimen repository. In the absence of an institutional data

Table 2. Examples of Biobanking Software Products

Product Name	Company	Contact
Sapphire	LabVantage (Bridgewater, New Jersey)	http://www.labvantage.com (accessed July 24, 2011)
Nautilus	Thermo Scientific (Waltham, Massachusetts)	http://www.thermoscientific.com (accessed July 24, 2011)
StarLIMS	StarLIMS (Hollywood, Florida, and Tel Aviv, Israel)	http://www.starlims.com (accessed July 24, 2011)
BIGR	HealthCare IT, Inc (Rockville, Maryland)	http://www.healthcit.com (accessed July 24, 2011)
caTissue Suite	National Cancer Institute (Bethesda, Maryland)	https://cabig.nci.nih.gov/tools/catissuesuite (accessed July 24, 2011)
BSI	Information Management Services, Inc (Rockville, Maryland)	http://www.bsi-ii.com/ (accessed July 24, 2011)

repository, such as an electronic medical record, data abstraction may be performed on a manual or semiautomated basis by using data entry technicians, who act as honest brokers and filter the electronic record before releasing data for research use with banked biospecimens. Manual methods naturally increase the costs of obtaining well-characterized biospecimens, whether for clinical or research purposes. Information technology support and integration of biobanking functionality into traditional hospital and laboratory information systems greatly facilitate the utilization of biospecimens for research, as many of these are primarily collected for clinical care.

A number of software vendors specialize in laboratory information management and specifically, in biorepository operations. Examples are given in Table 2. Some software systems focus on biospecimen tracking, while others focus on inventory management. Still others include clinical data annotation and strive to accommodate all aspects of the biobanking process. Direct integration with clinical care systems remains elusive because business models for these systems have traditionally not been aligned. This has only recently started to change, given new market forces such as the push for personalized medicine. Many commercial biobanking systems, once used, may be better able to track patient samples than existing clinical care systems, given the focus on biospecimens around which these systems are built. They may not provide for all the biobanking needs, and careful evaluation within a specific environment is required.

COST RECOVERY

Methods of cost recovery remain essential for the development and operation of sustainable biorepositories. When planning a biorepository, this process must include development of the underlying business model, including the identification of end users or “clients” who use the services and materials offered by the biorepository, budgets, and projected profit/loss and cash flow analyses. The biorepository director(s), in conjunction with senior management at their particular institution, should define potential sources of start-up funding, whether institutional, through grants from government or philanthropic entities, or from partnerships with industry. With this information, pricing models and associated fee schedules can be devised to enable appropriate recoupment of costs.

Different business models will be available to a biorepository, depending upon the setting in which it exists; for example, (1) serving clinical operations, (2) clinical and research services developed within an academic setting, or (3) a stand-alone business that may operate with or independently of academic or other National Institutes of Health (NIH)-funded entities.

Regardless of the setting, new biorepository resources should be considered start-up operations that will have a ramp-up period during which operations incur a loss as the business grows. Biorepositories should thus develop 3- to 5-year projections as part of their plan and determine the time frame in which operations will break even, or become profitable, or, in the case of academic/institutional biorepositories, assess if there will be an ongoing amount of subsidy from grants or other institutional sources to maintain operations.

Factors to consider for biorepositories operating in different settings include the following:

1. *Support of Clinical Operations:* Pathology departments generally incorporate costs for long-term storage of frozen and paraffin blocks within fees for clinical services provided. However, capital start-up costs associated with biorepository operations and supporting IT systems are material and need to be budgeted, in addition to the incremental operational costs. An active area of effort is the development of new current procedural terminology codes to charge third parties for the processing and storage of high-quality human biospecimens needed for the current generation of molecular tests. Costs to attain this level of quality frequently exceed the costs to store routine frozen and FFPE tissues. Many clinical sites thus partner with academic institutions or commercial entities to enhance their biorepositories, both for clinical purposes and to add research utilization of biorepository materials. The National Cancer Institute’s Cancer Human Biobank (caHUB) was recently formed in response to a critical shortage of high-quality, highly clinically annotated human biospecimens. This effort, based out of the Office of Biorepositories and Biospecimen Research, is currently developing evidence-based best practice protocols for all aspects of consenting, annotating, collecting, preserving, qualifying, storing, and distributing all types of human biospecimens, using literature review, consensus expert opinion, and contract-directed biospecimen research. These protocols will be available in the public domain as they are completed.
2. *Clinical and/or Research Operations in an Academic, Not-for-profit, or Government Setting:* Academic biorepositories commonly draw upon institutional support, direct NIH or philanthropic grant support (Specialized Programs of Research Excellence and related grants), as well as partnerships with commercial entities, to provide services. When developing a biorepository plan in an academic setting, it is important to define institutional contributions, which may come from indirect funds on grants to funded

investigators and may also affect amount of subsidy for costs to internal users. Biorepositories in academic centers that receive NIH funding are generally required to charge fees to internal users at cost, particularly NIH-funded investigators, which can limit the core's ability to expand or remain agile to new developments if the bulk of the core's budget comes from direct charge-backs to internal users. For this reason, grants or other forms of extramural support are often sought to undertake new projects or otherwise fund upgrading of infrastructure and equipment. Care must be taken to adequately fund IT infrastructure, including institutional support and maintenance for the infrastructure, as well as for biorepository operations. Most academic biorepositories thus retain 1 or more faculty members to write proposals for funding. Providing services to the commercial sector also can generate revenues to support improvements in the biorepository and assist with maintaining sustainable operations.

3. *For-profit Biorepositories*: Businesses basing their models on biorepository operations or support of biorepository operations in commercial or academic institutions also have the option of seeking angel and venture capital funding, in addition to grant resources, such as the Small Business Innovation Research and Small Business Technology Transfer grants, offered through the NIH. Many commercial entities also directly partner with commercial end users of samples, and with academic or nonacademic health care systems, to provide support for the development and maintenance of biorepository operations if individual sites lack the resources to undertake de novo development on their own.

CONCLUSION: BENEFITS TO THE COMMUNITY PATHOLOGIST

We emphasize that existing community pathology practices, which traditionally store biospecimens, have an excellent base for establishing biorepositories that can meet evolving needs for both clinical care and research purposes. Under the guidance of published best practices and standard operating procedures published by the government and other standard-setting organizations such as the CAP, laboratories may begin to make the transition to a viable high-quality biorepository service. Existing personnel who perform surgical consents, tissue triage, or specimen handling also can be leveraged for the collection and storage of high-quality biospecimens. Gaps in IT and other areas exist, but these are not insurmountable, particularly with recently developed free offerings that adhere to national standards published by the National Cancer Institute. Please refer to Table 3 for a collection of additional resources on guidelines, research, IT, and ongoing large scale initiatives.

The need for improvements in biospecimen quality and its associated clinical and laboratory information is increasing. For example, in anatomic pathology laboratories, stored FFPE blocks have historically aided in the care of patients at a very coarse, disease-specific level of granularity. Care for any single patient has been generally dictated by research-driven descriptions of the general disease processes across many patients, thereby missing nuances in the individual patient, which may be clinically significant for treatment. Because of the historic inconsis-

tency and variability in collection and storage of biospecimens, large data sets were required for drawing generalizations about disease processes with any degree of accuracy. The future will belong to those who seize the opportunity to standardize and improve procurement, storage, utility, and distribution of biospecimens to customize care for the individual patient. With improved biospecimen quality, the signal-to-noise ratio (the statistical significance of results) of downstream data obtained from biospecimen analysis increases, such that small variances can be analyzed rather than disregarded. This is the logical extension of current systems.

Pathology's involvement in biorepositories is a natural extension of its traditional role in biospecimen management. Vision, leadership, and administration in this area can position a pathologist as a valuable member of the medical staff and the local health system. The importance of quality biorepositories and quality biospecimens will become abundantly clear as our nation's health care system transforms to support an era of personalized medicine. Only proactive, forward-thinking pathologists will be adequately prepared to catalyze this transformation.

USE CASES

Tissue

The most common use case for tissue in the community setting generally focuses on remnant tissue from a diagnostic or therapeutic resection.

1. Institutional review board approval has been obtained and patient has properly consented to the collection of HIPAA-identified remnant tissue for on-site FFPE biorepository (diseased and normal tissue).
 - a. Specimen is rapidly transferred from surgeon to pathologist (pathology control) and cold ischemia time is documented.
 - b. Pathologist dissects the specimen in the operating room, or more commonly, rapidly transports the specimen to the gross room of the pathology laboratory for dissection. Appropriate transfer media is available in the operating room. This is critical for maintaining RNA integrity.
 - c. Tumor and normal slices are taken for formalin fixation within 60 minutes of the specimen being removed from the patient: "cold ischemia time."³
 - i. Two- to 3-mm thick slices are optimal.
 - ii. Ten percent phosphate-buffered neutral formalin *without* "proprietary additives."
 - d. Tissue slices are kept in formalin for 6 to 72 hours³ or 24 ± 8 hours (Clinical and Laboratory Standards Institute immunohistochemistry draft document currently in final voting) *including* the time in formalin during processing.
 - e. The pathology report contains the following times: cold ischemia time and total time in formalin.³
 - f. The redundant tissue blocks are stored or used for tissue microarrays per site-specific or national protocols.
 - g. The value of these specimens will depend upon how much clinical annotation is attached to the specimen and what type of patient follow-up information is attached to the specimen.
2. Same as above, but the blocks are deidentified ("anonymized") according to HIPAA. Institutional

Table 3. Additional Resources

Many of the critical steps to be followed in establishing and running a biorepository can be: 1) Modeled from fundamental guidelines that exist via policies and procedures found in Clinical Laboratory Improvement Amendments-certified laboratories and 2) implemented with guidance from industry best-practices to standardize and improve biospecimens and biorepositories. Organizations such as the College of American Pathologists (CAP), the Office of Biorepositories and Biospecimen Research and the International Society for Biological and Environmental Repositories provide such policies, procedures, and guidelines, in addition to third-party oversight such as the CAP Accreditation Program for Biorepositories. Additional resources and use cases related to specific types of biorepositories are summarized below.

Guidelines	<p>Betsou F, Luzergues A, Carter A, et al. Towards norms for accreditation of biobanks for human health and medical research: compilation of existing guidelines into an ISO certification/ accreditation norm-compatible format. <i>Qual Assur J</i>. 2007;11:221–294.</p> <p>College of American Pathologists. Informed consent for donation of biospecimens. http://www.cap.org. Updated September 3, 2010. Accessed July 24, 2011.</p> <p>International Society for Biological and Environmental Repositories (ISBER). Collection, storage, retrieval and distribution of biological materials for research. cell preservation technology. <i>Cell Preserv Technol</i>. 2008;6(1):1–56. http://www.isber.org/bp/BestPractices2008.pdf. Accessed July 24, 2011.</p> <p>Office of Biorepositories and Biospecimen Research. NCI best practices. http://biospecimens.cancer.gov/practices/default.asp. Accessed July 24, 2011.</p> <p>Organisation for Economic Cooperation and Development. Best practice guidelines for biological resource centres. http://www.oecd.org/dataoecd/7/13/38777417.pdf. Accessed July 24, 2011.</p> <p>World Health Organization International Agency for Research on Cancer. Common minimum technical standards and protocols for biological resource centres dedicated to cancer research. http://www.iarc.fr/en/publications/pdfs-online/wrk/wrk2/Standards_ProtocolsBRC.pdf. Accessed July 24, 2011.</p>
Research	<p>Karlson EW, Chibnik LB, Kraft P, et al. Cumulative association of 22 genetic variants with seropositive rheumatoid arthritis risk. <i>Ann Rheum Dis</i>. 2010;69(6):1077–1085.</p> <p>Lasky-Su J, Won S, Mick E, et al. On genome-wide association studies for family-based designs: an integrative analysis approach combining ascertained family samples with unselected controls. <i>Am J Hum Genet</i>. 2010;86(4):573–580.</p> <p>Wu AC, Himes BE, Lasky-Su J, et al. Development of a Pharmacogenetic Predictive Test in asthma: proof of concept. <i>Pharmacogenet Genomics</i>. 2010;20(2):86–93.</p>
Information technology	<p>Denny JC, Ritchie MD, Basford MA, et al. PheWAS: demonstrating the feasibility of a phenome-wide scan to discover gene-disease associations. <i>Bioinformatics</i>. 2010;26(9):1205–1210.</p> <p>Liao KP, Cai T, Gainer V, et al. Electronic medical records for discovery research in rheumatoid arthritis. <i>Arthritis Care Res (Hoboken)</i>. 2010;62(8):1120–1127.</p>

Table 3. Continued.

Initiatives	<p>Murphy S, Churchill S, Bry L, et al. Instrumenting the health care enterprise for discovery research in the genomic era. <i>Genome Res</i>. 2009;19(9):1675–1681.</p> <p>Ritchie MD, Denny JC, Crawford DC, et al. Robust replication of genotype-phenotype associations across multiple diseases in an electronic medical record. <i>Am J Hum Genet</i>. 2010;86(4):560–572.</p> <p>Roden DM, Pulley JM, Basford MA, et al. Development of a large-scale de-identified DNA biobank to enable personalized medicine. <i>Clin Pharmacol Ther</i>. 2008;84(3):362–369.</p> <p>Genomic Science Program. Human Genome Project information: ethical, legal and social issues. http://www.ornl.gov/sci/techresources/Human_Genome/elsi/elsi.shtml. Accessed July 24, 2011.</p> <p>National Cancer Institute. NCI's Cancer Human Biobank (caHUB). http://biospecimens.cancer.gov/cahub/default.asp. Accessed July 24, 2011.</p>
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review board typically waives consent for this use of remnant tissue specimens.

- a. Process is same as described above in section “1.”
 - b. These blocks are worth much less than those created in “1” above, because they have only limited non-PHI (protected health information) annotation attached to the specimen and no follow-up medical information. However, they are valuable for many types of pilot research and validation studies.
 - c. Billing will be the same as described in section “1,” if appropriate.
3. Institutions doing transplants: remnant or nonused specimens.
 - a. These are institution-specific situations and protocols, but creating, processing, storing, and transferring processes should be the same as described in section “1.”
 4. Routine and nonroutine (rapid) autopsies.
 - a. Routine autopsy tissue is not suitable for most molecular analyses other than some DNA studies.
 - b. Rapid autopsies are not applicable to the community pathology laboratory.

Research

Human biospecimens used for research may be (1) dedicated samples, collected from consented patients or (2) discarded/excess materials that were collected in the course of clinical care. The use of samples and associated clinical data is governed by federal laws including the Common Rule (45 CFR 46) and the HIPAA Privacy Rule. Regulations at the state level and from other federal agencies, including the US Food and Drug Administration and NIH, may govern the collection and utilization of human biospecimens for research purposes.

The Department of Health and Human Services defines research as “a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge.”² A further expansion of this definition by the NIH states:

Research conducted with human subjects or on material of human origin such as tissues, specimens and cognitive phenomena, for which an investigator or colleague directly interacts with human subjects. Excluded from this definition are in vitro studies that utilize human tissues that cannot be linked to a living individual. Patient oriented research includes: (a) mechanisms of human disease, (b) therapeutic interventions, (c) clinical trials, and (d) development of new technologies.

Per the above definitions, research may use excess/discarded clinical materials that have previously been collected in the course of clinical care. Use generally requires review by an IRB regarding utilization of samples and any associated clinical data. Samples are commonly released in 1 of 3 states:

1. An anonymous data set is associated with data so limited that it cannot readily be linked to a given person. Furthermore, it would remain exceedingly difficult to reidentify a particular individual even if the researcher attempted to compare the anonymous data set against a separate identified store of the original clinical data. While statistical methods can be used to determine the likelihood of possible reidentification, it necessarily depends upon the amount of anonymized clinical data linked with the sample, compared to the original data set, as well as the size of the population from which a sample may have originated. For practical purposes, many biorepositories treat age and gender as a sufficiently anonymous data set, except for patients older than 90 years. Biorepositories are otherwise recommended to review what additional data they would consider releasing as part of an anonymous data set, through an internal review process that should include consultation with their local IRB.
2. A deidentified data set comprises a spectrum of data sets. At the more stringent end, a fully deidentified data set is one in which all 18 HIPAA identifiers have been removed, including conversion of dates to year only and zip codes to first 2 or 3 digits, removal of free text reports, and obfuscation of numeric values. Other forms of deidentified data sets include "limited data sets" for which partial or full date and time stamps may be included, such as date of birth or death and dates/times of clinical procedures, and/or geographic information, including town, city, or full zip code associated with a given event. Coded data sets are controlled by a third party, termed the *honest broker*, who may have access to the original identified data set and derive a deidentified data set that is provided to the researcher; alternatively, in the case of biorepositories managing consented samples, the biorepository derives a deidentified data set from its consented data set to be provided to a researcher who does not require access to identified patient data. In both cases, the honest broker retains a coded link back to identified patient data even though the researcher only has access to information that has been deidentified.

Anonymous and deidentified samples and data sets must at a minimum meet the requirements stipulated in the Department of Health and Human Services and NIH definitions of research, namely, associated data must be in a form that does not identify a given living person.

Sites providing anonymous or deidentified samples for research use commonly have investigators and associ-

ated study staff sign a "data and sample use agreement" that stipulates terms under which the provided samples and data may be used and that researchers will not attempt to reidentify subjects, on the basis of their research or any access to clinical records that they may have, in the course of their research or in the course of delivery of medical care. Biorepositories providing anonymous and deidentified biospecimens are also recommended to work with their local IRB to codevelop standards and procedures for the collection and release of materials. These efforts will ensure appropriate oversight and compliance as defined within individual state or institutional guidelines.

3. Consented data sets are derived from biospecimens with patient informed consent for use of their excess or discarded biospecimens and clinical data. Identifiers may remain linked to materials, generally in a secure, password-protected database or other secure system. The physical sample should not have protected health information printed on labels; rather, a unique sample identifier (sometimes dual or multiple identifiers) is used; these values do not directly represent clinical identifiers.

While consented biospecimens are clearly needed for long-term follow-up and prospective outcomes-based studies, it should be noted that the costs and infrastructure to obtain consented biospecimens are frequently an order of magnitude or more above those required for deidentified and/or anonymous collection. Research studies may thus structure their studies for use of consented biospecimens for certain aspects requiring long-term follow-up and monitoring of outcomes, and may use deidentified or anonymous samples for analyses that either require minimal linkage to clinical data or suffice with a deidentified data set.

With these factors in mind, each of the abovementioned types of samples has particular uses. Anonymous and deidentified materials are frequently used in the development and validation of clinical assays and research based on retrospective events that do not require long-term outcomes or linkage with the clinical record. Large population-based studies using deidentified data sets linked to discarded samples have powered many studies evaluating risk factors for common diseases such as hypertension, diabetes, and asthma. The i2b2 NIH roadmap initiative (Informatics for Integrating Biology to the Bedside; <https://www.i2b2.org>, accessed July 24, 2011), in particular, has developed tools that enable ethical use of large discarded biospecimen sets linked to deidentified or consented data sets.¹³

Microbes/Clinical Isolates

Tests for infectious agents are the most commonly encountered molecular assays performed in pathology practices. Testing detects the presence of pathogen-specific DNA or RNA signatures. The widespread use of viral load testing for human immunodeficiency virus and other RNA-based viruses has driven the development of procedures in CLIA-certified laboratories regarding optimal collection and processing of fluid samples for detection of such agents.

Pathologists are advised to be aware of state-specific recommendations or mandates regarding diseases to be reported to departments of public health and appropriate collection of patient samples that may be required for

confirmatory testing or subtyping of strains identified in the primary clinical laboratory. Examples include strain typing of samples testing positive for influenza and serotyping of reportable enteric pathogens.

Regarding the use of clinical isolates for research purposes, free virions, bacteria, fungi, or parasites are not considered human tissue, though they may have originated from human samples. Unless detailed patient data are provided with samples, use of these materials does not necessarily require IRB review, although sites are advised to make certain recipients have certification to handle pathogens categorized as biohazard level 2 (BL-2) or other levels of biohazard. Isolates provided with information from the clinical record, which may be considered more than an anonymous data set, do require IRB review regarding research use of the associated information.

Cells and Fluids for Genetic and Genomic Studies

Completion of the first sequenced human genome in 2003 provided a new framework for evaluating an individual's genetic makeup to identify variants that contribute to disease or predispose toward a particular disease. Efforts in subsequent years have focused on defining "normal" genomic variation across populations. This accumulated information provides a necessary base for identifying and using variants that cause diseases or otherwise predispose individuals to the development of a given condition.

While pathologists have long been involved in genetic testing, we should expect changes to the practice of pathology that incorporate methods for analyzing significant portions, or all, of a patient's genome. These analyses will differ from currently performed testing in a number of ways, including the following:

1. Need to store and analyze orders-of-magnitude more information.
2. Development of standards for classifying and evaluating polymorphisms and other structural variants as contributory to a given disease process.
3. Multiplex analyses to be able to analyze alterations at multiple loci to help define risk or otherwise aid in diagnosis and management.
4. Need for standards to undertake intermittent interrogation of a patient's genetic data for clinical triggers from annual routine health visits to visits associated with specific medical events.
5. Development and use of clinical decision-support tools that enable interpretation of complex genetic traits against published evidence as it accumulates.
6. Billing processes to support additional diagnostic support in these areas as rendered by pathologists and supporting staff.

For clinical workups that include genetic and/or genomic evaluations, patients are commonly asked to sign clinical consent forms for the testing. This consent differs from research consent, though many centers include forms and documentation to the patient to cover both activities. The clinical consent may detail the potential for future clinical analysis and reporting of findings in their genetic information as new information comes to light, with appropriate medical evidence, supporting the interpretation of polymorphisms and other

structural genetic alterations. Frequently, these updated interpretations occur in the context of future clinical visits, or as requested by a physician managing the patient's care.

Importantly, given the rise of "recreational genomics" in recent years, genetic and genomic testing for clinical purposes must be performed in a CLIA-certified laboratory that has necessary standard operating procedures in place for (1) validating the clinical significance of newly identified mutations and (2) communicating updated results to patients and their physicians unless stipulated differently for research purposes.

The pathologist can be expected to play an increasing role in these areas as many multitrait tests, on the cusp of moving into clinical practice, need significant input of clinical/phenotypic data to provide results with high predictive values. The reference laboratory performing the testing may not have required access to the patient record, but the local pathologist would, thus allowing enhanced interpretation of diagnostic information to clinicians, particularly when it combines additional test results that were obtained in the local laboratory and/or pathology practice.

While these areas continue to rapidly evolve, we anticipate that multitrait tests for common diseases, such as diabetes, asthma, and cardiovascular disease, may be among the first to be handled in community settings. While research methods commonly use broad sequencing or gene-array analyses, the combined data may be more effectively implemented in a CLIA setting, on existing platforms for molecular diagnostics that assay a panel of single-nucleotide polymorphisms or targeted probes for structural variants such as deletion and insertions. In fact, these types of assays have been developed for evaluating mutations associated with cystic fibrosis and for human immunodeficiency virus genotyping. Furthermore, environmental factors, such as diet, behavioral factors, and other types of exposure, significantly influence a variety of complex, polygenic disorders. Thus, biospecimens, in addition to those used to prepare DNA, will need to be collected for a complete diagnostic evaluation. As an example, genetic predictors for development of type 2 diabetes, or responsiveness of persons with type 2 diabetes to various therapeutic modalities, would likely be evaluated in the context of the patient's fasting glucose and hemoglobin A1c levels.

When it becomes more cost-effective to sequence an entire patient's genome, versus an assay for deleterious mutations at defined locations per immediate medical need, the community pathologist will become a central part of the team in community health care systems to define how this information is stored, accessed, integrated, and used for clinical purposes. With this in mind, we note that pathology residency programs increasingly need to include hands-on experience during which trainees have the opportunity to work with complex data sets, regarding how they are generated, managed, and used for the delivery of evidence-based medicine.

While many practitioners find these developments daunting, it should be noted that the practice of pathology has always focused on the analysis of complex sources of information to provide clear diagnostic interpretation and guidance to clinicians and patients. This core tenant of pathology will not change. As the methods and techniques for obtaining diagnostic information have evolved over time, so should we view these developments as another

step in the practice of pathology. They will provide yet another opportunity for us to provide quality care and innovation in the delivery of health care.

This effort reflects a white paper initiative commissioned by leadership of the College of American Pathologists. Many thanks to those who provided feedback, including (in alphabetical order) Mahul Amin, MD; Tarek Bishara, MD; Steve Black-Schaffer, MD; Phil Branton, MD; Alexis Carter, MD; Sam Caughron, MD; Thomas Cooper, MD; James Crawford, MD, PhD; Richard Friedberg, MD, PhD; Philip Ginsburg, MD; Steve Gutman, MD; Mark Hanley, MD; Andrew Horvath, MD; Jill Kaufman, PhD; Debra Leonard, MD, PhD; Carl Morrison, MD; Jonathan Myles, MD; Shuji Ogino, MD; David Rimm, MD, PhD; Aaron Sassoon, MD; Gail Vance, MD; Jim Vaught, PhD; and Louis Wright, MD. Supported by NIH grant R01-LM-010100 (Dr Bry).

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