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The NCI Biospecimen Research Network

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Abstract

High quality cancer research using human biospecimens must be based on biospecimens that have been obtained according to rigorous ethical and procedural standards. Methods for biospecimen collection, stabilization, processing and storage for research, however, are highly variable and the biospecimens available for research often are of unknown quality. The National Cancer Institute Biospecimen Research Network program was initiated in 2006 to conduct, sponsor, report and collaborate on research to better understand the effects of different biospecimen collection methods on downstream molecular analysis. An online Biospecimen Research Database and an annual symposium, *Advancing Cancer Research through Biospecimen Science*, have been developed and many research projects are underway to develop a knowledge base from which to develop evidence-based biospecimen standard operating procedures and methods for assessing biospecimen quality. These efforts will enable better cancer research and development efforts.

Key words: best practices, biobank, biospecimen, cancer, human, quality, research

High quality research is attained by using high quality human biospecimens

It is essential that the cancer research enterprise generate high quality research and clinical data. When human biospecimens form the basis of research studies, it is important that researchers and clinicians utilize high quality biospecimens. It has become clear in recent years, however, that significant time, effort and research dollars are expended in producing research that is based on biospecimens of uncertain quality. The classic adage of "garbage in, garbage out" becomes especially problematic when poor quality biospecimens form the basis of important research studies (Compton 2007).

But what defines a "good quality" biospecimen? What defines a "garbage" biospecimen? To answer these questions one must better understand the biological state of the biospecimen, how it is influenced by various preanalytical factors, and how these influences may alter downstream research.

The biological state of the human biospecimen reflects its stress status and disease status

In the model for translational cancer research, biospecimens from cancer patients are assumed to reflect the disease biology. Research is performed on biospecimens obtained from cancer patients, and researchers and clinicians hope to apply what is learned in the laboratory to new developments in cancer diagnostics and therapies to benefit patients. But in this model, what often is not considered is that a "real" biospecimen has unique biology owing to the biological stress that the specimen undergoes during collection, processing and storage procedures. In the National Cancer Institute's (NCI) Biospecimen Research Network (BRN) program, the unique biology of the biospecimen is the object of investigation.

The NCI's Office of Biorepositories and Biospecimen Research (OBBR) launched the BRN in

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response to concern about the issue of biospecimen biology. The lack of high quality biospecimens has been identified as the number one obstacle to cancer research and the use of biospecimens of poor or unknown quality can compromise the accuracy of data derived from research funded by the NCI. This issue must be addressed to move cancer research and development forward. The issue relates not just to government, academics, or industry, but to all sectors involved in the cancer research enterprise and to cancer patients. The multitude of issues related to the quality and availability of biospecimens for cancer research was the impetus for establishing the OBBR in 2005.

OBBR's approach to improving the quality of human biospecimens used for research

The OBBR's approach to improving the quality of human biospecimens used for research is threefold. The first approach is the development of the NCI Best Practices for Biospecimen Resources. The Best Practices provide state-of-the-science guidance for biobanking to harmonize procedures for collection, processing, storage and distribution of biospecimens (http://biospecimens.cancer.gov/bestpractices). The second approach is to sponsor and conduct research through the BRN program to understand better how pre-analytical variables affect the molecular integrity of the biospecimen (http://biospecimens.cancer.gov/researchnetwork/default.asp). OBBR now is developing a third approach to integrate information from the Best Practices and the BRN into new, evidence-based biospecimen Standard Operating Procedures (SOPs) to facilitate cancer research.

The NCI Best Practices for Biospecimen Resources was first issued in 2007. The Best Practices outline the operational, technical, ethical, legal, and policy best practices for NCI supported biospecimen resources. They incorporate key principles that define the state of the science with regard to biospecimen resource practices, promote biospecimen and data quality, and support adherence to ethical and legal requirements. A newly revised version of the Best Practices was posted recently for public comment. The comments will be incorporated in the coming months and OBBR will issue a new version. The Best Practices now are in a web-based format at the OBBR web site (http://biospecimens.cancer.gov/bestpractices).

Need for biospecimen research

The BRN supports further development of Best Practices for collection, annotation, processing and storage based on scientific evidence. To develop this program, starting in 2006, the NCI explored the evidence already available about how different biospecimen collection, processing and storage procedures affect biospecimen molecular integrity to determine what new research is needed.

The area of biospecimen research is complex because many pre-analytical variables can affect the molecular integrity of the biospecimen. These pre-analytical variables can be encountered prior to acquiring biospecimens (pre-acquisition phase) and after the specimen is collected (post-acquisition phase). Pre-acquisition variables may include antibiotics that the patient may be taking, other drugs that the patient may be taking, type of anesthesia that may be administered during a surgical procedure, duration of anesthesia, and arterial clamp time during surgery. There are many post-acquisition variables as well, which can include the time at room temperature prior to biospecimen stabilization, temperature of room, type of biospecimen preservative, time in preservative, rate of freezing of biospecimens, method of freezing, and size of aliquots. The lists above are by no means comprehensive. Medical and surgical procedures, biospecimen handling and processing, storage of biospecimens, distribution, and restocking of unused sample all can affect the molecular integrity of the biospecimen (Fig. 1).

In genomics, it is known that changes in specific transcript levels can be observed that are related to ischemic time, not disease (DeCecco 2009). With regard to arterial clamp time, which defines how long a tissue has been deprived of oxygen during surgery before it is removed from the body, it is known that significant changes in gene expression occur owing simply to changes in clamp time (Schlomm 2008). These changes are not related to disease, but rather to pre-analytical perturbations of the biospecimen.

In proteomics, it is clear that lack of reproducibility of protein biomarkers in discovery research is due partially to the lack of standardization of the biospecimens used for these studies and the lack of qualification of the specimens for very high throughput, highly sensitive technologies (Rai 2005). It also is known that the detection of proteins in tissues by immunohistochemistry can be affected greatly by various biospecimen pre-analytical variables, which cause inconsistent immunohistochemistry results

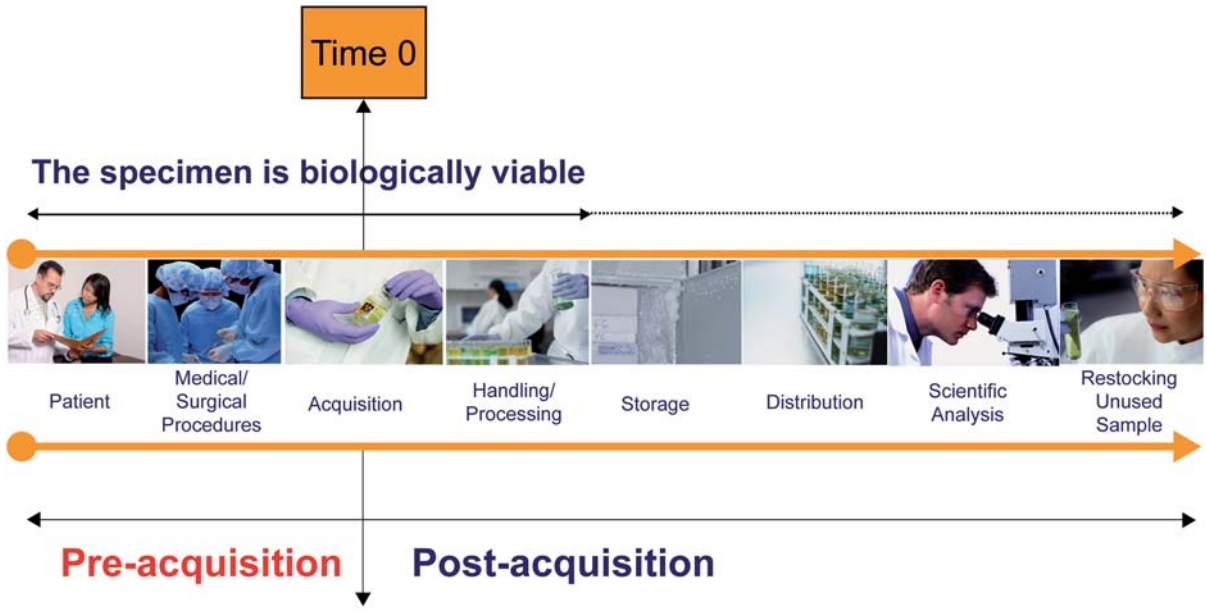

Fig. 1. The life cycle of the biospecimen.

in research and clinical laboratories (Williams 1997, Engel and Moore, 2011).

In the area of metabolomics, changes in biospecimen molecular integrity could cause inconsistencies in small molecule readouts (Barton 2008), which yields results that point to the wrong pathway.

The NCI BRN program

The BRN supports collaborative research in the highly complex area of biospecimen science. This is accomplished by providing a forum for research results on how biospecimen variables affect molecular analysis, generating new research data through BRN extramural research programs, and multiple collaborations with other programs in the US and internationally to facilitate biospecimen research and evidence-based biospecimen practices. The BRN provides a forum for research results through the freely available online Biospecimen Research Database and the annual BRN symposium, *Advancing Cancer Research through Biospecimen Science* (http://biospecimens.cancer.gov/meeting/brnsymposium/).

The goal of the Biospecimen Research Database is to make existing and emerging biospecimen research data more accessible to a wide range of researchers. The field of biospecimen science has been active for many years but is not a high profile area of investigation. Publications in this area are found in many journals, which makes this type of data difficult to access by the genomic and proteomic researchers who really need to know this information. Recognizing this need and the need to assess the type and strength of existing data to guide future research initiatives, the BRN designed an online database that is expertly curated to contain summaries of such hard-to-find research papers. The Biospecimen Research Database can be found online at http://biospecimens.cancer.gov/brd. Using different search capabilities, a user can locate literature summaries on diverse topics related to how specific biospecimen collection, processing, and storage procedures are related to the outcome of molecular studies. For example, a paper in the database reports that genome-wide genotyping using FFPE tissue is reliable and produces results that are reproducible with frozen specimens (Lips et al. 2005). This is an important finding to consider for those working in genomics who may have access to FFPE tissues for research. The Biospecimen Research Database now has 600 papers from many journals (Fig. 2).

The annual BRN symposium, *Advancing Cancer Research through Biospecimen Science,* had its fourth annual meeting in March 2011. The symposium is a widely attended scientific meeting that last year attracted more than 400 attendees and 100 attendees via live videocast. The symposium features speakers from around the world on the subject of biospecimen science and gives attendees the opportunity to engage in interactive discussions, learn about recent advances in biospecimen science, and attend workshops on biospecimen quality (Moore et al. 2009). More information can be found at http://biospecimens.cancer.gov/meeting/brnsymposium/. The archived videocast

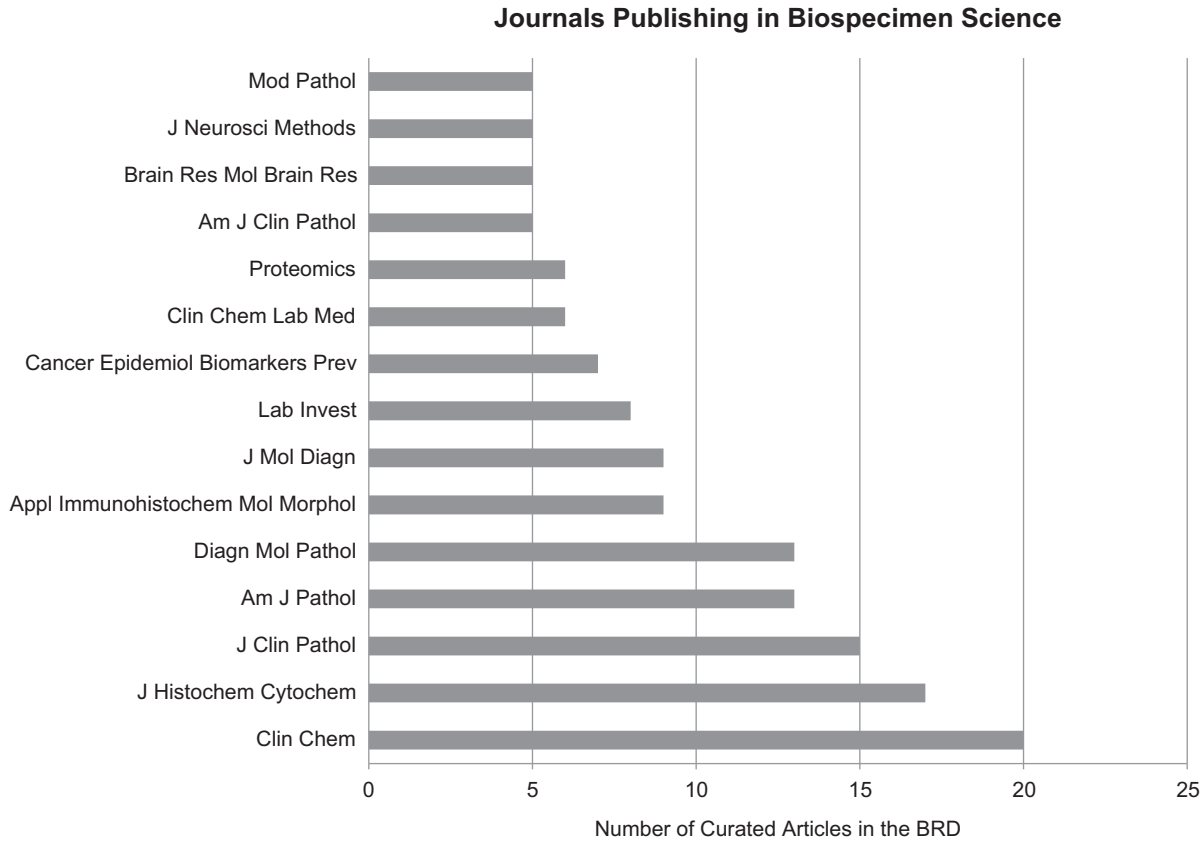

Fig. 2. Distribution of biospecimen science publications in the Biospecimen Research Database.

and presentations from previous years are posted on the web site, as are a number of tools that help to communicate the importance of these issues.

New BRN-sponsored research

The BRN generates new research data through its extramural research programs and collaboration with the existing NCI Innovative Molecular Analysis Technologies (IMAT) program in "Innovative and Applied Emerging Technologies in Biospecimen Science" (http://innovation.cancer.gov).

The BRN extramural research program was designed around three central themes. First, "bridging the gap" between existing clinical practice for biospecimens and emerging technologies for personalized diagnostics and therapies; second, defining the most significant variables for prospective collection of tissues, blood, and body fluids; and third, developing evidence-based biospecimen quality indicators for specific analytical platforms. Research topics within these central themes include:

- Tissue preservation variables and their impact on downstream applications. (The effects of tissue preservation variables on the results of HER2/neu testing in breast cancer tissues is an example of this issue in the context of companion diagnostics.)
- Effects of pre-acquisition variables and biomolecule extraction methods on biomolecule analysis results in blood.
- Assessment of whether a banked specimen is suitable for a specific molecular analysis approach.

The BRN research program, entitled, "Biospecimen Research for Molecular Medicine," was developed with two major aims: 1) to develop innovative approaches to control, monitor and assess biospecimen quality and 2) to define systematically the impact of key pre-analytical variables in specific types of human biospecimens on downstream molecular data generated from specific molecular analysis platforms. The first aim is served by investigator-driven contract research for which requests for proposals (RFPs) are issued on the topic of "biospecimen molecular integrity." For the research contracts under this program, biospecimen collection and molecular analysis procedures

must be known and reproducible, and annotation and quality control are paramount. The research contracts in progress under this program include:

- Investigations into the effects of blood specimen handling procedures on protein integrity (Dan Chelsky, Caprion)
- Credentialing plasma and serum biospecimen banks for proteomics analyses (Katherine Williams and Susan Fisher, University of California, San Francisco)
- Intrinsic and extrinsic controls for FFPE tissue (David Rimm, Yale)
- Effects of biospecimen integrity, intratumoral heterogeneity, and analytical variance on microarray-based pharmacogenomics tests of breast cancer (W. Fraser Symmans, MD Anderson and Christos Hatzis, Nuvera Biosciences)
- Effects of pre-analytic variables on circulating microRNAs (Hua Zhao, Roswell Park)
- Rapid methods for the assessment of tissue quality (Charles Saller, ABS)

To accomplish the second aim, the BRN has designed a program aimed at understanding the variability introduced by different tissue fixation and processing procedures. The program is a multi-site study designed to establish the state of practice for cancer tissue collection, processing and storage; to study the effect of different practices on biospecimen molecular integrity; to focus on the procedural variations that cause the most significant variations in molecular integrity; and to use these data to inform the development of evidence-based standard operating procedures (SOPs). The BRN currently is constructing the experimental design for this program and is interested in identifying robust ways of analyzing the tissues to reveal the molecular changes induced by pre-analytical variables such as the time to fixation, length of fixation, and different processing parameters. The findings from this new research will be incorporated into new, evidence-based biospecimen practices.

Opportunity for biospecimen science

Enormous opportunities exist for advancement in the field of biospecimen science including enabling better, more reproducible cancer research; developing better products from that research faster, better, and more reliably; and translating better research into better products for patients. OBBR and the BRN, together with the broader research and development community, can accomplish this by building a new knowledge base in biospecimen science through new research and communication of new and existing research findings through publications, meetings, and the Biospecimen Research Database.

It is also important to change how research using human biospecimens is published. A community effort led by the BRN is working on a project called, "Biospecimen Reporting for Improved Study Quality (BRISQ)" (Moore et al., 2011). The basic idea is that researchers must say more in their publications about where the biospecimens that form the basis of their study came from and how the biospecimens were treated. BRISQ is a complement to existing reporting recommendations such as STROBE and REMARK (von Elm 2008, McShane 2005) and could have a large impact on making research results more reproducible.

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