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Quality Bio-Specimens for Novel Biomarker Discovery

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Introduction

Recent advances in genomics, proteomics, and biotechnology have provided unprecedented opportunities for translational research and personalized medicine. The data that can currently be gathered from human bio-specimens can be used to detect diseases and identify molecular mechanisms and therapeutic targets. Thus, high-quality specimens annotated with relevant clinical information and collected with scrupulous attention to ethical, legal, and social concerns are integral to reliability of study results.

This article outlines the specific requirements that must be met in order to obtain highest quality bio-specimens while following strict bioethical standards. The goal of increasing awareness on this issue is to facilitate generation of valuable research outcomes.

Bioethical Standards

It is critical that specimens utilized in a study are obtained under approved IRB/EC protocol with proper informed consent from study participants [1]. Informed consent should be designed to present potential human research participants with sufficient information, including anticipated procedures, risks, and benefits, so that the participant can make an informed decision as to her/his involvement in the research study.

To assure that informed decisions are made, the consent form should use clear language to explain research objectives in layman's terms. The form should address the intended use of bio-specimens and/or collected data as well as the possible development of tests and other commercial products that may result from the researchers' discoveries. The document should also include the investigator's agreement covering confidentiality, use, disposition, and security of bio-specimens and associated information [1].

To protect confidentiality and comply with federal patient privacy regulations, all clinical samples collected for research should be de-identified. De-identification requires removal of all identifiers and/or codes that could allow the researcher to trace the sample back to the donor. This process requires stripping of all identifying information and the assignment of a unique research number to the patient. This research number should then appear on all data collection forms and clinical samples. In this way, each specimen, along with its associated data, would thus be linked to a particular patient ID number and tracked throughout its lifetime in the repository [2].

Protocol Standardization

Standardization of collection, processing and storage protocols is integral to the production of bio-specimens suitable for clinical research. Standardized protocols (SOP's) should be applied consistently to ensure bio-specimen quality and to avoid introducing variables into research studies. The collection and processing protocols have to be based on authoritative best practices and/or solid research data, adapted from published methods, or developed by in-house technical experts. All SOP's should be made available to potential researchers before they obtain the materials for their studies.

Collection and Storage of Frequently Used Human Biospecimens

Blood specimens

Blood allows for molecular detection a spectrum of genetic and physiological events, which can be associated with various diseases and conditions. Its informative value, along with its ease of collection (a minimally invasive procedure) makes peripheral blood is an attractive source for biomedical research. Samples collected *in vivo* are best for these types of studies and the amount of blood drawn and the types of collection tubes used vary depending on the researcher's specific objectives [3-7]. Three types of blood samples are commonly collected, utilizing special tubes for identification: Serum sample (BD Vacutainer with red cap), Plasma sample (BD Vacutainer with purple cap), and RNA sample (PAX gene RNA tube). The Serum sample is then fractionated into serum and clot, with serum being used for analysis of blood proteins and clot used for DNA analysis [8-10]. The Plasma sample is fractionated into plasma and cell fractions with Plasma used for analysis of blood proteins and identification of plasma associated DNA and Cell fraction is used for cell-associated DNA studies [11-14]. The whole blood, collected in RNA PA Xgene tubes is used for the isolation and stabilization of RNA [15,16].

The Serum and Plasma samples are fractionated into their components using a centrifuge. The components are then aliquotted into cryovials and placed into a -80°C freezer. The whole blood, collected in RNA PAX gene tubes is incubated at room temperature for at least 2 hours to allow complete lysis of blood cells before processing or freezing. The SOP's for processing of these peripheral blood samples should describe the appropriate supplies to be used, centrifugation speed and time, aliquot volumes, storage temperature, and shipping specifications.

Tissue specimens

Most tissue samples are collected from patients undergoing surgery or diagnostic procedures. As with blood RNA, RNA in tissue is susceptible to ischemia-induced injury and can rapidly degrade upon removal from the patient [17-19]. Therefore, it is imperative that the tissue is processed and frozen as soon as it is surgically removed. For quality assurance purposes, the warm ischemia interval (the time period based on when the tissue sample is removed from patient to the time of cryopreservation) should be carefully monitored. Once the sample arrives in the laboratory, the pathologist with specialized training

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examines the specimen in the fresh state and records all relevant pathological information. It is good practice to harvest adjacent grossly normal tissue in addition to lesional tissue (e.g., tumor). Tissue is harvested for research purposes only after pathologist has determined that all diagnostic requirements have been satisfied. The prioritization of patient diagnosis over collection of specimens for research purposes is vital to ensuring that patient care is not compromised.

Different preservation methods should be used for different molecular analyses of tissue samples. Formalin fixation provides excellent preservation of tissue morphology, and makes bio-specimens useful for a variety of applications including targeting of proteins [20], *in situ* hybridization [21,22], etc. Although well suited for diagnostic pathology laboratories, formaldehyde fixation induces extensive protein and nucleic acid cross-linking and makes recovery of biomolecules tenuous at best. These samples are not satisfactory for high-through-put expression methodologies such as cDNA microarrays or two dimensional polyacrylamide gel electrophoresis (2D-PAGE). Therefore, a crucial need exists for processing methodologies that produce good histological details and also allow recovery of mRNA and protein of sufficient quality and quantity for molecular profiling studies. OCT (Optimal Cutting Temperature) – this approach not only retains tissue morphology, but allow investigators to perform high-through-put molecular analysis on all of the various biomolecules in a sample.

Tissue is heterogeneous. Each single section of tissue can contain multiple cell types. Laser microdissection is a technology that allows cells of interest to be isolated from a tissue-mounted slide, thus creating a pure cell population. Molecular studies can then be performed reducing contamination by bystander cells and producing more reliable results. OCT-embedded tissue is especially well adapted to this technique because it allows the tissue to be sectioned directly on the microtome and provides good histological detail.

Alternatively, tissues can be frozen directly in Liquid Nitrogen. These flash frozen specimens can be converted into OCT sections if required or can be utilized for studies that do not require histological analysis [23,24].

Storage conditions depend on research type for which the samples will be used. There is no consensus on optimal storage conditions. Storage for frozen specimens (OCT embedded or snap frozen ranges from -80°C to -190°C in the vapor phase of liquid nitrogen. Lower temperature may help preserve molecular integrity of specimens for long-term storage. FFPE tissue blocks and tissue microarrays should be stored under conditions that protect them from extremes of temperature or humidity.

Documentation and biorepository database

Thorough documentation of bio-specimen collection, processing, storage steps and conditions is essential to the quality of research data. The best approach is to document all relevant information (e.g. time to banking, time of ischemia, time of bio-specimen excision, character of chemical preservation, time of fixation, etc.) in a computerized inventory-tracking database. Storage conditions should also be recorded, with special attention given to deviations from SOP's, including information about temperature, thaw/refreeze episodes, and equipment failures. Data in the database has to be maintained for an appropriate period of time, in a standardized format and available for distribution as needed. The database must include original and current quantities of bio-specimens, dates of sample exhaustion, sample locations and bio-specimen movements within or out of the bio-repository. All data must be stored securely and protected against unauthorized access. Commercially available software, e.g. Freezerwors from Data works Development, Inc., or proprietary databases could be utilized for this purpose.

Clinical data is the most important information this database should contain. Bio-specimens should be linked to clinical data in compliance with the HIPAA and HHS and FDA human subjects protection regulations. A basic dataset of clinical and pathological data should be recorded for each bio-specimen. Annotation of specimens should ideally include donor ID and donor demographics, primary and additional diagnoses, primary cancer site and anatomical site where the specimen was collected, tumor morphology, behavior (i.e., benign, malignant), grade, and pathological stage. H&E stained slides should be reviewed to ensure accurate and consistent pathological data, eliminate problems associated with observer error, and control the overall quality of tissue samples.

Quality control

Quality control (QC) is the essential finishing step in the processing of bio-specimens. Tissue QC involves H&E slide preparation and pathological evaluation to insure tissue identity and histology (Figure 1). If the specimen is intended for use in Molecular biology studies, RNA extraction and subsequent evaluation using the Agilent 2100 Bioanalyzer and RNA 6000 Pico Labchip kit (Agilent Biotechnologies, Palo Alto, CA, USA) (Figure 2) tissue samples is the method of choice for confirming the bio-specimen quality [25,26].

Human bio-specimens are integral to any successful translational

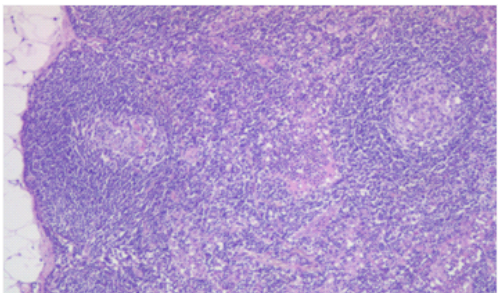

Figure 1: H&E slide normal mesenteric lymph node.

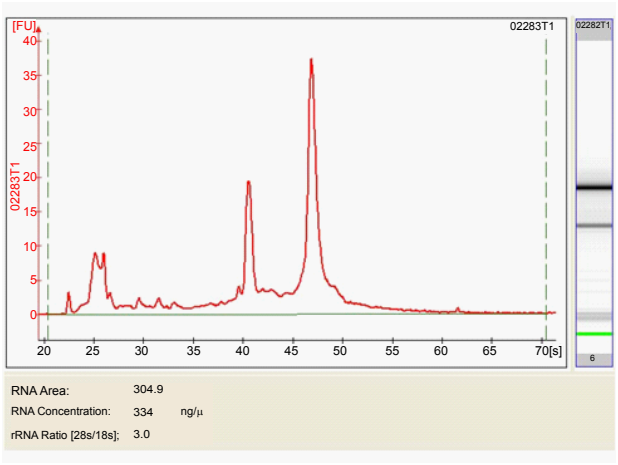

Figure 2: Agilent 2100 Bioanalyzer ferrograph.

clinical research program. Access to human bio-specimens that are well-defined, carefully collected, and obtained with proper consent provides researchers with unique patient and disease-specific resources that complement traditional research on cell lines and mouse models. Attention to detail at all levels of bio-specimen acquisition will result in an invaluable resource for the research community.

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