

The NHLBI Biorepository

Guide to Building Biospecimen Collections for Study and Future Research Use



Contents

1.	The NHLBI Biorepository	3
1.1.	Background	3
1.2.	Responsibilities of the Biorepository	3
1.3.	Benefits of Participation	4
1.4.	Important Considerations for Applicants	5
1.5.	Requirements for Biorepository Applicants	6
2.	Planning to Build a Biospecimen Collection.....	7
2.1.	Overview.....	7
2.2.	Budget Planning	7
2.3.	Staffing Expertise	7
2.4.	Facilities and Equipment	8
2.5.	Biospecimen Inventory System Software.....	9
2.6.	Transition Plan	9
3.	Designing a Collection.....	9
3.1.	Overview.....	9
3.2.	Human Subject Considerations.....	10
3.3.	Materials to Preserve	10
3.4.	Pre-analytic Variables.....	11
3.5.	Number of Vials/Aliquots to Store.....	12
3.5.1.	Aliquot Scheme.....	12
3.5.2.	Aliquot Volume.....	13
3.6.	Label Design.....	14
3.6.1.	Label Stock and Label Validation.....	15
3.6.2.	Label Information	16
3.7.	Storage Configurations.....	18
3.8.	Biospecimen Associated Data.....	18
3.9.	Assay Data.....	18
3.10.	Transition Planning.....	19
4.	Ensuring Biospecimen Quality.....	19
4.1.	Overview.....	19
4.2.	SOP Structure and Definitions	19
4.3.	QA and QC Plans	22
4.3.1.	Training and Competency	22
4.3.2.	Quality Control	23
4.3.3.	Quality Monitoring	23
4.3.4.	Pilot Studies.....	23
5.	Study Documents.....	24
5.1.	Overview.....	24
5.2.	Document Library.....	24

1. The NHLBI Biorepository

1.1. Background

The National Heart, Lung, and Blood Institute (NHLBI) Biologic Specimen Repository (Biorepository) serves as a shared scientific resource consisting of archived biospecimen collections from NHLBI funded clinical studies (Studies). In its role as custodian, the Biorepository has served as a valuable scientific resource for almost four decades by acquiring biospecimen collections from NHLBI funded Studies and distributing those specimens to qualified investigators at no fee beyond shipping costs. Over the years, these collections have been used to make seminal contributions to public health in establishing the origin and transfusion transmissibility of several viral agents and addressing new scientific questions in heart, lung and blood diseases. Access to biospecimens is through the NHLBI Biologic and Data Repositories Information Coordinating Center (BioLINCC) website at <https://biolincc.nhlbi.nih.gov/>.

This guide is designed to assist NHLBI Studies that are considering building a biospecimen collection suitable for Study and/or future use by the broader research community. Each chapter includes details that can facilitate the planning and collection activities necessary to acquire biospecimens that are fit for the purpose intended and maximize future scientific utility. Studies should regularly refer to this Guide throughout the active Study phase to ensure that the processes used for sample acquisition and preservation are appropriate and effective.

1.2. Responsibilities of the Biorepository

The Biorepository is actively involved in all phases of the acquisition and maintenance of biospecimen collections from NHLBI funded studies. Experienced staff members are available to assist and consult in the planning, monitoring and transfer processes of creating biospecimen collections for archive.

The Biorepository also plays a key role in the review of applications for new specimen collection submissions as described in [Section 5.0 of the BioLINCC Handbook](#). For example, following preliminary acceptance of the Study data in Data Repository a subset of the specimens are physically relocated to the Biorepository as a pilot shipment. Selected specimens undergo QC checks, including inspection of vials and data for the following elements:

- Vial label information matches data files.
- Locations of materials within shipment(s) and cryoboxes are recorded accurately
- Vial/container types are appropriate and consistent.
- Material characteristics (e.g., liquid volume, hemolysis) are captured in the data files.
- The physical inventory is consistent with the Study documentation provided with the application.

These quality checks are performed to ensure that the biospecimens are suitable for request, shipment and use by future researchers, and to ensure that any discrepancies or inconsistencies can be resolved prior to opening the collection to the scientific community.

The results of the pilot shipment QC are provided to the NHLBI along with a recommendation for inclusion/exclusion and an estimated budget for transfer of the full collection. Upon receipt of the full collection, additional QC is performed using the same criteria used for the pilot shipment.



Figure 1: Flow chart of specimen transfer and vial scanning

In addition, the Biorepository is also responsible for cataloging pre-analytical information about the collection so that future researchers are able to determine if the specimens will be suitable for the assays they will perform as part of their research.

Once a collection is “opened,” the Biorepository maintains the collection, evaluates requests, and makes recommendations regarding the suitability of the specimens for proposed future research. The Biorepository is responsible for shipping logistics and distribution of biospecimens for approved research requests.

1.3. Benefits of Participation

Applications to transfer a new Study Collection into the Biorepository will be considered for any study funded by the NHLBI. There are numerous benefits for having a biospecimen collection accepted into the Biorepository, including:

- Valuable specimens obtained through research efforts are made available to the wider scientific community.

- Subsequent research using shared biospecimen resources must acknowledge the parent Study source in publications.
- Long-term storage of collections is provided in an established facility, at no charge to the researcher.
- All biospecimens are inventoried and tracked in a secure centralized database, including the creation and disposition of any subsequent aliquots.



Figure 2: Tasks at each stage of bulk specimen transfer to the Biorepository

1.4. Important Considerations for Applicants

Building a biospecimen collection to serve as a resource for qualified investigators in the wider research community requires careful and deliberate practices when acquiring the specimens as well as adequate resources to build, store and sustain a collection when the Study's primary funding ends. The processes and procedures used to execute a Study protocol may not be sufficient to build a robust collection with future scientific utility. Given that research questions are not defined when a collection is transferred to the Biorepository, all collections undergo a rigorous evaluation process to ensure that the biospecimens and linked data have potential scientific utility and that funds are available to transfer the collection. An application process is used to facilitate this evaluation that includes examining the overall completeness of the Study and biospecimen-associated data, the quality and number of the biospecimens proposed for transfer, the ability to link data with individual vials, the ability to electronically transfer all data, the availability of funds to transfer the collection, the potential cost of maintaining the collection, and the potential future scientific uses.

It is important that applicants know that transfer of a collection to the Biorepository is also a transfer of custodianship of the collection. The NHLBI becomes the caretaker of the collection and assumes responsibility for maintaining the collection. Custodianship also includes the rights to determine conditions under which the biospecimens are accessed, used, and retained. Information on submitting an application and accessing biospecimens is provided in the BioLINCC Handbook.

The Biorepository only accepts biospecimens via bulk transfer after the Study has been completed and the collection is ready for open access by outside researchers. Incremental transfers (shipments) during the active Study collection phase are only considered in exceptional circumstances.

1.5. Requirements for Biorepository Applicants

Studies that are considering applying to transfer a collection to the Biorepository should first ensure that the collection can meet the following Requirements:

- The application must be sponsored by the Study's NHLBI Program Official.
- An Institutional Review Board (IRB) must have reviewed and verified that submission of the collection to the Biorepository for subsequent sharing with non-Study investigators for research purposes is consistent with the informed consent of Study participants.
- The Study must provide a biospecimen electronic inventory file in the format available in the [NHLBI Biospecimen Collection Questionnaire](#) and a complete curated Study data set that links to the inventory file at a vial level. See [Chapter 4.0 of the BioLINCC Handbook](#) for information on preparing data sets. Biospecimens are not accepted at the Biorepository until the data sets have been submitted, reviewed and approved by NHLBI.
- The Study must provide a completed [NHLBI Material Transfer Agreement \(MTA\)](#), indicating that the Study is willing to transfer custodianship of the biospecimens and Study data to NHLBI, that an IRB certifies that the biospecimens may be shared with non-Study investigators and that the Study acknowledges that that collection size may be reduced in the future if the biospecimens are determined to have no/low scientific utility.

In addition to the requirements stated above, collections that do not meet the following key considerations are unlikely to be accepted. While special accommodations may be made for older and unique collections that do not meet these requirements, Biorepository resources are limited and only collections with high scientific utility can be supported.

- Uniqueness of the collection: The future scientific utility of a collection is based primarily on its potential to be used. If there is an abundance of biospecimens already available to researchers that have similar clinical, demographic and sample attributes then additional biospecimens with these characteristics may not be needed.
- Rich, complete clinical data: The clinical data linked to a biospecimen is the single most important factor in its selection for potential research. If biospecimens cannot be linked to complete and accurate data records, then they will not be used.
- Broad Consent: Informed consent documents must allow for research by the scientific community. Each restriction placed on the use of biospecimens limits its potential for sharing.
- Data on biospecimen quantity: Collections must record accurate quantities (volume, mass, etc.) for each vial/biospecimen in the inventory. Nucleic acid specimens should also have the concentration provided. Using a default estimated volume or concentration for all vials irrespective of the actual volume is not appropriate. A central laboratory or biorepository has to know what physical resources are available for research.
- Biospecimen documentation: The Study documentation has to provide evidence that the material types being submitted are suitable for future scientific use. If it is not possible to determine the conditions under which a biospecimen was collected, processed and stored, then the collection cannot be effectively promoted to researchers.

- Barcodes and unique IDs: Managing non-barcoded vials requires considerable extra resources at the Biorepository. Locating vials in a collection that contains barcoded labels but where that barcode is not unique to the vial causes considerable confusion and additional work to access and distribute. Manual inventory checks and handling of vials increases the labor required to house and distribute the collection.

Subsequent chapters provide information on the essential components needed to build a robust Study collection to serve the both the current Study and be a scientific resource for future research.

2. Planning to Build a Biospecimen Collection

2.1. Overview

Establishing a biospecimen collection for Study and for future use requires careful and deliberate planning and adequate resources to fund and support biobanking activities. Key elements for consideration include:

- Budget planning.
- Staffing expertise.
- Facilities, equipment and software.
- Transition planning.

2.2. Budget Planning

Financial resources are needed to create a quality Biorepository collection. Budget planners must consider supporting the cost of facilities including experienced staff that will be required during the active phases of the Study as well as support the activities needed after the Study has ended. Studies must budget for the people, facilities, and time needed to collect, annotate, process, monitor, and store additional biospecimens for future use during the course of the trial. Study budgets should include the costs of Study close-out activities, preparing for transfer by assembling the data, and consolidating biospecimens that will be retained for future research. Finally, they should plan on the cost for the physical transfer of the collection at the end of the Study to a new location (e.g., courier cost, freezers, and racks).

2.3. Staffing Expertise

Many clinical studies, especially ones with multiple research centers, use a central laboratory to perform the assays dictated by the clinical protocol. Typically, additional biospecimens are stored by the central lab and these are the subset of biospecimens that would be submitted as a collection to the NHLBI and used for future study. Care must be taken to ensure that staff at the central laboratory have expertise in preparing a collection for future research in accordance with published [best practices, guidelines and standards](#). Similarly, Study coordinating centers must have the ability and expertise to build adequate IT infrastructure to track and monitor biospecimen locations, processing, handling and associated data.

In addition to having clinical, statistical and assay expertise, there must be staff with expertise in designing, building and maintaining a biospecimen collection. This includes:

Staff qualified to:

- Prepare Standard Operating Procedures (SOPs) for sample and data collection, sample preservation and inventory control.
- Implement the regulations for proper transport of biospecimens.
- Develop an appropriate Informed Consent Form (ICF) that allows participants to approve future use of their biospecimens and clinical data by non-Study investigators.

Staff with experience and training in:

- Biospecimen collection, processing and preservation techniques.
- Collection, collation, analysis, and quality control of the data associated with participants and their biospecimens.
- Building and implementing quality assurance and quality control (QA/QC) plans to ensure that studies are conducted properly and that all aspects of biospecimen and data collection are completed as the trial is executed.
- Preparing electronic inventory files of the biospecimens that efficiently link to clinical, demographic and assay data.



Figure 3: Biorepository staff accessing freezer

2.4. Facilities and Equipment

There must be adequate facilities to collect, process, store and maintain the collection. Adequate and appropriate freezer space will be required to house the collection. In addition, equipment and staff must be available to provide round-the-clock temperature monitoring and to keep records of equipment maintenance and repairs. Storage equipment often includes ultralow temperature units (e.g., vapor phase of liquid nitrogen [LN₂ at -180°C] or -80°C freezers) and adequate dedicated backup storage units. Samples should be stored in a manner that allows for efficient transfer of the stored specimens in the event of a freezer failure or major temperature fluctuation.

2.5. Biospecimen Inventory System Software

A validated and robust electronic biospecimen inventory system capable of tracking biospecimens at a vial level is essential. Inventory systems can be developed in-house or purchased commercially. The BSI inventory system used by the Biorepository is a commercial product that is configured to capture collection, processing, shipping, and storage information associated with each individual vial. Any electronic inventory system used must go through an initial qualification process, must be capable of tracking each specimen (“parent”) with any subsequent aliquot or processed derivative (“children”) and must be capable of electronically documenting vial data in a format that can be merged with Study clinical data.

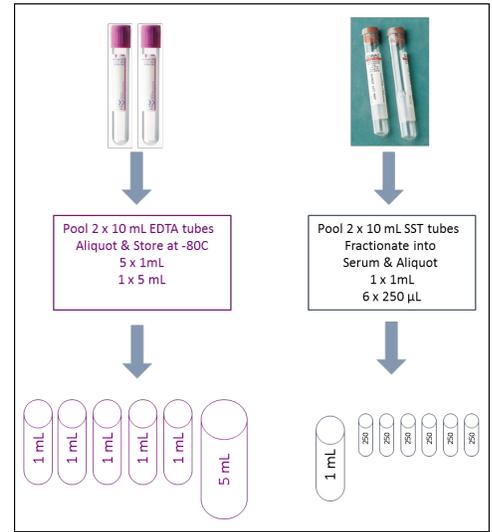


Figure 4: Examples of specimen lineage flow from collection to aliquot

2.6. Transition Plan

The transition plan should include consolidation of Study documents, assembly of data, and efficient organization of the biospecimens to be transferred. The applicant will be responsible for making shipping arrangements and paying for all associated shipping costs.

Collections accepted through the application process may receive planning assistance from the Biorepository.

3. Designing a Collection

3.1. Overview

Determining the material types (whole blood, serum, plasma etc.) to collect, the data to collect and record for each specimen, and how to label the samples requires deliberate planning and expertise. Design of the collection must take into account both the intended Study use and the potential future use of the biospecimens if the plan is to use collection for non-Study research. Studies should only consider building and sharing a new collection with the Biorepository if they have planned in advance to have the adequate resources and expertise necessary to support such an effort.

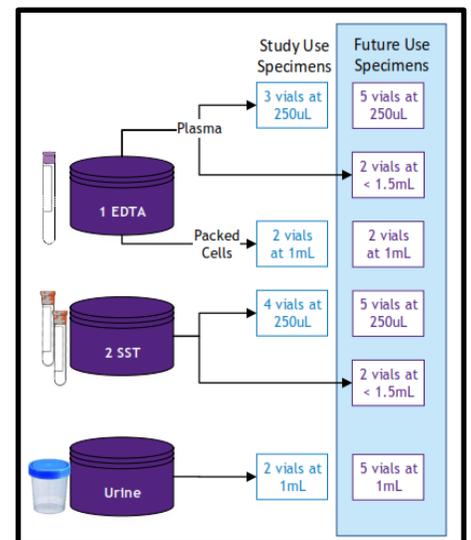


Figure 5: Specimen collection/aliquot scheme: Study Use vs. Future Use

This chapter reviews the elements to be considered when designing a collection. The Study's NHLBI Program Official may request assistance from the subject matter experts at the Biorepository by contacting the NHLBI Biorepository Contracting Officer Representative (COR).

3.2. Human Subject Considerations

Study participants who donate biospecimens must be informed about the use of the biospecimens and who will have access to them, both now and in the future. If the intent is to make the biospecimens and data available for future research to non-Study investigators, then the Study informed consent document should state this unambiguously. It should also address the period of time that the biospecimens are expected to be stored and what may happen to the biospecimens when the Study closes.

Collections transferred to the Biorepository must have an electronic data file that links each specimen to the informed consent data regarding its future use. If the consent document does not include clear intent to share the biospecimens in the future and it is decided later to do this, then the Study group will need to obtain IRB approval. Studies also complete a Material Transfer Agreement (MTA) confirming that biospecimens can be shared with non-Study investigators. The signed MTA acknowledges transfer of custodianship of the collection to NHLBI, and the potential for reduction of the collection if not used.

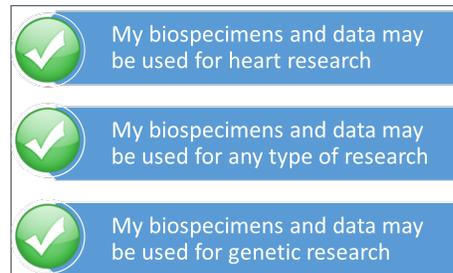


Figure 6: Examples of multiple choice consent types

The Office of Human Research Protections (OHRP) has a variety of policy and regulatory guidance materials and helpful tools available on its website to aid the research community in the conduct of ethical research: <https://www.hhs.gov/ohrp/regulations-and-policy/index.html>

3.3. Materials to Preserve

The types of biospecimens collected will depend on the downstream assays to be performed. These assays therefore determine many of the specimen collection data parameters. For example, if the purpose of collecting the sample is testing using a PCR assay, then the material type and its collection, processing and storage methods are defined for that assay in the Study-specific procedures. When designing a collection for future use, the Study Protocol should include procedures to collect sufficient material quantities. The collection plan should also be designed to address ancillary studies and potential future research needs.

For example, certain assays may specify or exclude

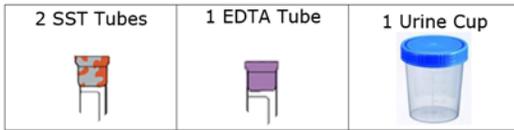


Figure 7: Examples of specimen collection container types and numbers for a collection plan

- Material types (whole blood, serum, plasma, etc.)
- Anticoagulant (EDTA, Na-Heparin, ACD, etc.)
- Extraction/processing method prior to analysis
- Exposure to freeze/thaw cycles

If any such assays are reasonably anticipated as future research possibilities, samples of the appropriate type should be drawn and preserved for the future during the course of the Study.

3.4. Pre-analytic Variables

Pre-analytic variables are sample variables that are known to affect assay results. These variables can have an impact on the scientific utility of a biospecimen. For example, biomarker proteins are often labile and are therefore susceptible to inconsistencies in handling and processing. When planning a Biorepository collection, consider capturing the following common pre-analytical variables in the data set. Documenting this information is a necessary part of following Best Practice for Biorepository specimens.



Figure 8: Study lab staff processing collected specimens

- Specimen collection techniques
- Preservatives used
- Processing methods
- Processing materials and reagents
- Quantities required
- Vials/containers (volume, type and material)
- Storage conditions
- Freezing and thawing methods
- Exposure to Freeze/thaw cycles (number and timing of events)
- Temperature and time elapsed at each handling/processing step

The ISBER Biospecimen working group developed a SPREC tool to capture some of the most common pre-analytical variables in a standardized code list. SPREC codes are used at the Biorepository for both historical and contemporary collections. See the table below for examples of SPREC codes associated with a whole blood sample:

Pre-analytical Variable	Type of Sample	Type of Primary Container	Pre-centrifugation (Time & Temp)	Centrifugation	2 nd Centrifugation	Post-centrifugation Delay	Long-term Storage
Description Example	Blood (Whole)	Potassium EDTA	<2H, 2 to 10°C	2 to 10°C, 10 to 15 min, <3000g with Brake	No Centrifugation	<1 h RT	Cryotube, 1mL to 2mL, (-85) to (-60)°C
SPREC Code ⁽¹⁾	BLD	PED	A	D	N	B	D

⁽¹⁾ **Standard Preanalytical Coding for Biospecimens: Review and Implementation of the Sample PREanalytical Code (SPREC)**, Lehmann et.al. Biopreservation and Biobanking. August 2012, 10(4): 366-374. doi:10.1089/bio.2012.0012.

Another highly valuable resource tool when considering the storage of blood samples, is a blood draw tube guide. CLSI has published guidelines that contain advice for common assay types and Becton Dickinson (BD) provides an illustrated listing of the different types of vacutainers they make, their typical uses and collection requirements. While this guide is vendor specific, the information can be applied to tubes from any vendor.

https://www.bd.com/vacutainer/pdfs/plus_plastic_tubes_wallchart_tubeguide_VS5229.pdf

3.5. Number of Vials/Aliquots to Store

Single-use frozen aliquots are valuable because the biospecimens have never been thawed. These aliquots are considered “pristine.” The cost and effort to aliquot a previously frozen biospecimen is greater than the cost to aliquot before the biospecimen is cryopreserved. The number and size of aliquots must balance the need for pristine biospecimens with the practical considerations of freezer space and storage costs. In the current experience of the Biorepository, approximately 95% of the assays performed on collections in our care are not affected by 1-2 freeze/thaw cycles. Of note, the actual vial content, e.g., the volume or concentration of the material in the vial, should be recorded in the Study’s electronic data files for each vial. A default volume or concentration should not be used.

3.5.1. Aliquot Scheme

The Study must develop a collection visit schedule and determine the aliquot scheme for each visit and material type. The Biorepository typically recommends a scheme that has between 3-5 single use pristine vials and additional vials with larger volumes to conserve space. Additional aliquots can be created at a later date from biospecimens that are in high demand, and for renewable resources (such as cell lines and DNA) as these can be expanded at a later date. Table 3.1 below contains the most common single use aliquot size and aliquot schemes for various whole blood derivatives.

Aliquot schemes can vary widely based on the diversity and volume of materials to be collected. Study groups anticipating submitting biospecimens should consult the Biorepository prior to setting their aliquot scheme.

Table 3.1: Example of aliquot schemes for whole blood derivatives.

WHOLE BLOOD DERIVATIVE	EXPECTED YIELD FROM A 10 ML BLOOD TUBE (~8.5 ML OF BLOOD)	COMMON SINGLE USE VOLUME OR QUANTITY	NUMBER OF MICROCRYOVIALS
Serum	3.5 to 4.5 mL	.25mL	4 vials @ 0.25 mL remainder at 1 mL/vial
Plasma	3 to 4 mL	.25mL	4 vials @ 0.25 mL remainder at 1 mL/vial
PBMC	5 x 10 ⁶ to 20 x 10 ⁶ cells	5 x 10 ⁶ cells	Viable Cells: 5 x 10 ⁶ /vial Pellets: 1 x 10 ⁶ /vial
DNA	240 µg from Fresh Blood 200 µg from Frozen Blood	2µg	4 vials @ 2 µg remainder in 2 vials
RNA	12 µg	1µg	4 vials @ 1 µg remainder in 2 vials

3.5.2. Aliquot Volume

The impact of freeze-thaw cycles on the clinical biospecimens should be considered when determining the aliquot volume and the expected yield for each aliquot of a particular material type. Tables 3.2 and 3.3 provide average expected yields for various derivatives based on the experience of the Biorepository. Manual extraction methods typically provide slightly higher yields when performed by experienced staff, but automated methods tend to be more consistent and offer higher throughput.

The yields reported in the tables below are provided as a general guideline. The exact methods used by the Study laboratory will determine the actual yields. For a particular study with pre-determined test plans, the aliquots designated for those assays should be created immediately after the blood is drawn to minimize freeze-thaw cycles. In general, the aliquot scheme for vials planned for future research should be designed to produce a combination of single use and larger volume aliquots.

Packed cells intended for later DNA extraction are typically stored in only two vials (one for the initial extraction process and one as a backup) since DNA derived from this material is the target and can be divided into multiple aliquots after extraction and quantification.

For urine, the most common assay volume required is 1 mL. Single use tubes of urine should be stored at this volume.

Of note, the actual vial content, e.g., the volume or concentration of the material in the vial, should be recorded in the Study's electronic data files for each vial. A default volume or concentration should not be used.

Table 3.2: Average DNA Yields

MATERIAL TYPE (STARTING VOLUME OF SOURCE MATERIAL)	EXTRACTION METHOD	AVERAGE DNA YIELDS (μg)
Whole Blood (10 mL) Fresh	Manual Puregene	240
Whole Blood (10 mL) Fresh	Autopure	260
Whole Blood (10 mL) Frozen	Autopure	200
Packed Cells	Autopure	240
Buffy coats (8-10 mL blood)	Autopure	140
Blood clots (10 mL)	Phenol/Chloroform	260
Buccal Mouth wash (5 mL)	Phenol/Chloroform	21
Buccal Mouth wash (5 mL)	Manual Puregene	24
Buccal Brushes (2)	Manual Puregene	6.4
Lymphocytes (5 million cells)	Manual Puregene	21
Serum (0.4mL)	Manual Puregene	0.75
Serum (0.4mL)	Qiagen	0.53

Table 3.3: Average RNA Yields

MATERIAL TYPE (STARTING VOLUME OF SOURCE MATERIAL)	METHOD	AVERAGE RNA YIELDS (μg)
Whole Blood (2.5mL) ¹	Qiagen/BD PAXgene	3
Purified PBMC (10 x 10 ⁶)	RNeasy	5
Purified PBMC (10 x 10 ⁶)	Tri Reagent	10

¹Note that some of the most commonly used collection vessels for RNA are the same size as the 10 mL blood collection tubes but only hold 2.5 mL of whole blood. For example, the RNA PAXgene[®] tube holds only 2.5 mL of whole blood so the expected recovery would be 3 μg of RNA, not the 12 μg in Table 3.1.

3.6. Label Design

Labeling refers to both label stock (the physical label and adhesive placed on a biospecimen vial/container) and the label information (the text and barcodes printed on that label). Each Study must develop a labeling/coding plan for uniquely identifying each vial/sample and to maintain the link to the Study participant data. The Biorepository requires that each vial or sample be electronically linked to the Study Participant ID codes, the donor's Informed Consent limitations and the storage location (Freezer/Rack/Box/Row/Column). If some of the vials/samples in a

collection are not consented for use by non-Study investigators then these vials must be removed from inventory prior to transferring the collection into the Biorepository.

Non-barcoded labels, labels with handwritten information and labels with private participant information are generally not accepted for transfer to the Biorepository. In addition, each vial must have a unique coded identifier. Vials without unique identifiers require additional resources to inventory, retrieve and distribute and are only accepted in the Biorepository if the collection is unique and has potential scientific value, and if resources are available to support the additional level of effort required to manage them.

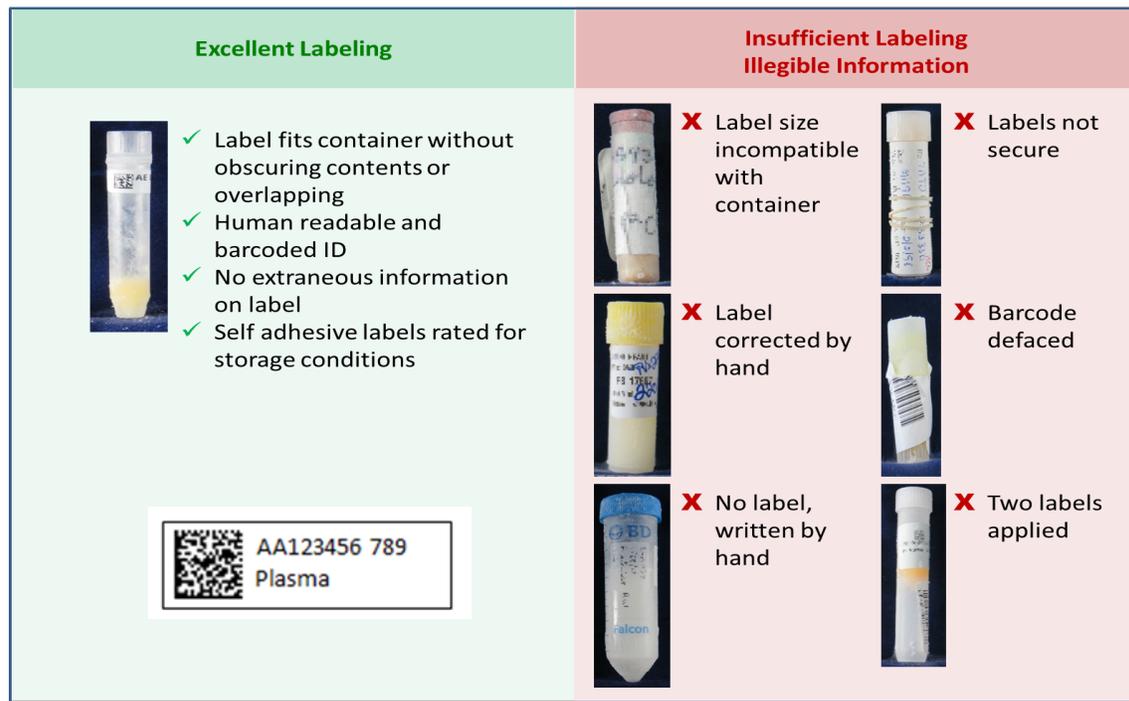


Figure 9: Example of a well-labeled vial and barcode vs. examples of insufficient or illegible labeling

3.6.1. Label Stock and Label Validation

The physical labels applied to biospecimens must be tested to ensure that labels applied to the Study-specific sample storage containers will remain readable and remain adhered to the containers throughout its expected life. Adhesives can become brittle when frozen causing labels to fall off, and labels are often subject to damage over time. Labels used for long-term storage in ultra-cold units such as -80°C freezers and liquid nitrogen freezers (-180°C) must be specifically designed and tested to withstand these conditions.

In addition, biospecimens used for molecular applications may be exposed to even greater extremes of temperature. For example, the vials may be submerged in water baths (100°C) for rapid thawing in the lab, heated and cooled to extremes, and/or routinely wiped with alcohols and

other solvents to decontaminate the external surfaces. Some cell or tissue biospecimens may be best preserved if they are snap frozen, requiring the biospecimen to go from room temperature to -65°C in seconds, and then transferred to a liquid nitrogen freezer to reach storage temperatures close to -196°C .

Any labels used should be tested to make sure they remain adhered and readable under the planned conditions of the parent Study, its testing facilities and at the long-term storage temperatures that the Biorepository will use. The label should fit on the vial without obscuring vial contents and inks must be permanent. The labels also should be usable and readable under a variety of foreseeable types of downstream applications/testing that the Biorepository and future researchers may use.

The Biorepository does not recommend using a complicated labeling scheme. Choose a sturdy cryogenic label stock and use the same label configuration on all vials for the Study.



Figure 10: Photos of various specimen cryotube types

3.6.2. Label Information

Each label should include a unique coded identifier in both human readable and barcoded format. A brief description of the biospecimen material type in the vial (i.e., serum, PBMC, packed cells, plasma, buffy coat, etc.) may also appear on the vial label to assist laboratory personnel during processing and handling. No other information (e.g., donor identifiers, dates, volumes, etc.) should be present on the vial label and no information should be handwritten on the vial label.

The barcode should be tested to ensure it can be scanned reliably by all parties that will be handling the specimens. If there are multiple bar-codes on a vial (e.g., label barcode and the bar-code built into the vial), then both bar-codes should be scannable and both should be included in the Study data sets.

A common labeling scheme is one in which all biospecimens from a single collection time point (e.g., Study visit) are assigned the same root ID. A sequence number that denotes the aliquot created from that collection time point is then added making the specimen vial unique.

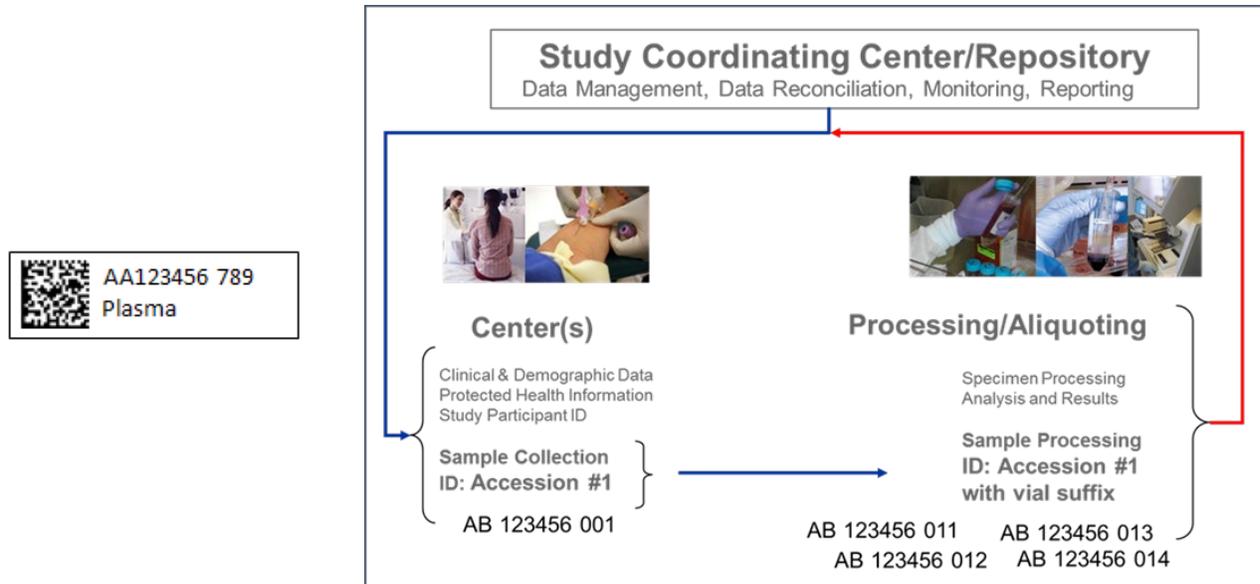


Figure 11: On the left, an example label with a 2D barcode, unique coded vial identifier and material type. On the right is an illustration of the use of the Sample ID to identify the specimen draw, and the vial suffix sequence numbers to identify aliquots created from that draw.

The BSI inventory database currently used by the Biorepository uses a coded identifier called a BSI ID that is unique to each vial. The BSI ID is made up of two components: an accession number that is unique to the draw of a participant (Sample ID), and a sequence number that is unique to the vial within that draw (multiple blood tubes drawn the same day, multiple aliquots created from one sample). Below is an example of the standard label used by the NHLBI Repository:

In this example, AA123456 is the Sample ID and 789 is the sequence number. All vials created from a single specimen collection would be labeled with the same Sample ID, but the sequence number on each vial generated from that collection makes the vial identifier unique. An example full BSI label set can be found at https://biolincc.nhlbi.nih.gov/media/guidelines/label_set.pdf

The information on the biospecimen labels is reviewed as part of an application to transfer a collection to the Biorepository. Studies must be able to provide an electronic inventory using a unique identifier for each vial/specimen in the collection. Labels should not include personal identifying information such as a participant's name, initials, social security number, date of birth, medical record number, or any other information that would potentially enable an investigator to ascertain the identity of the person to whom that sample belongs. Only in exceptional circumstances will biospecimens that are not labeled with a unique identifier and/or a unique barcode be accepted in the Biorepository. Specimen labels must not contain hand-written information and label stock must adhere to the vials during routine handling.

3.7. Storage Configurations

Biospecimens should be stored in a manner that reduces risk and in the most overall efficient configuration. A common risk mitigation strategy is to split aliquots of the same material into different storage units. Efficiency evaluation should take into account the level of effort when placing specimens into inventory as well as the effort required when retrieving samples. Retrieval is often more time consuming and should be given the greatest consideration when determining an optimal scheme.

In the experience of the Biorepository the most efficient storage configuration is to group specimens by material type and to split aliquots between at least 2 storage locations for risk mitigation purposes as well as for subsequent inventory management at the Biorepository.

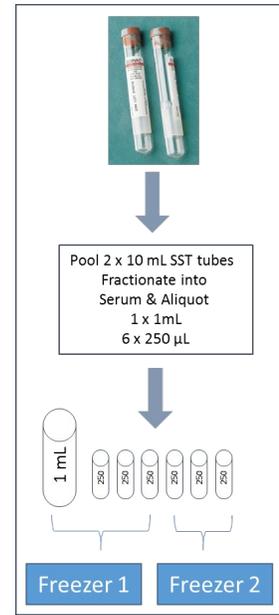


Figure 12: Example of a storage configuration across two freezers of a pooled serum draw

3.8. Biospecimen Associated Data

Data elements for collection, processing and storage should be recorded for each biospecimen to provide non-Study researchers with the information needed to determine the suitability of the biospecimen for their intended research use. A list of these elements can be found in the [NHLBI Biospecimen Collection Questionnaire](#). These data should also be electronically captured and linked to the Study data, including the informed consent restrictions. If these data are not collected, the scientific utility of the collection is limited and may result in the application to transfer the collection to the Biorepository being denied.

Additional data elements describing the processing procedures used on each specimen should also be collected at a vial or Study level. For example:

- Centrifugation parameters
- Pre-centrifugation delay – the time elapsed between collection and processing
- Post-centrifugation/pre-freeze delay – the time elapsed between centrifugation and freezing
- Storage container/vial type/preservatives

3.9. Assay Data

A description of the assays performed on biospecimens in the original Study is of vital use for future researchers to help determine the suitability of a sample for the intended research. These data are also used to assess the future scientific utility of a collection. The data elements recommended by the Biorepository for assays that should be captured at a vial and/or Study level include:

- Analyte measured.
- Commercial kit/part number/manufacturer or lab-developed assay used.
- The platform and version number used.
- Pre-analytical sample preparation method.
- Assay validation.
- Limit of detection.
- Percent coefficient of variation.
- Linear range.
- Unit of measure.
- Expected reference range.
- Quantitative definition of descriptive results (i.e. anti-HBs non-reactive = anti-HBs levels less than 8.5 mIU/mL, anti-HBs reactive = anti-HBs levels greater than 11.5 mIU/mL).

3.10. Transition Planning

The initial design of the Study should include a long term plan for what will happen to the biospecimen collection and data after the Study has been completed. Submission of the biospecimens and data to the Biorepository should be considered but, given that not all collections are suitable or accepted by the Biorepository, alternate plans should be established if long-term storage is envisioned.

A transition plan should include consolidation of Study documents, assembly of data, and time to efficiently organize and sort the biospecimens that will be transferred.

4. Ensuring Biospecimen Quality

4.1. Overview

Standard procedures and Quality Control (QC) and Quality Assurance (QA) plans should be established and implemented when building a biospecimen collection. QC is the procedure of examining a process, product, document, biospecimen or test result to ensure it meets a minimum level of quality. QA is the systematic monitoring and evaluation of various aspects of the QC process to maximize the probability that established standards are being met.

4.2. SOP Structure and Definitions

Standard Operating Procedures (SOPs) must be developed for all activities which will occur multiple times during the Study. As previously stated, these SOPs should be followed uniformly at all collection sites and at the processing and testing laboratories. Specific elements that are commonly present in an SOP are outlined below.

Table 4.1: Common elements addressed within SOPs

SECTION	DEFINITION	COMMENTS
Revision History	Revision number, approval date and summary of changes	<p>SOPs must be approved by qualified laboratory and quality personnel before use.</p> <p>If approvals are not an integrated part of the completed SOP, then provide information about where to locate approvals.</p> <p>SOPs may change over time to incorporate new knowledge. The revision history allows researchers to track changes and determine differences between biospecimens handled under different revisions of the SOP.</p>
Purpose	Describes why the processes described in the SOP are done	Usually the first section of an SOP.
Scope	Describes the boundaries of the process	Usually immediately follows the Purpose.
Responsibilities	Defines the department or job title of the people performing the tasks described in the procedure	May cover multiple departments and personnel, such as laboratory technicians, supervisors, and quality staff.
Materials	Lists the materials and supplies needed to complete the procedure	<p>Item descriptions should be detailed enough for future researchers to determine the exact materials used.</p> <p>Processing materials can affect the biospecimens (i.e. trace metal analysis is not indicated for specimens stored in contact with silicone because it can leach into the plastic).</p> <p>Best practice is to include the manufacturer's catalog numbers for all materials used.</p>

SECTION	DEFINITION	COMMENTS
Equipment	Defines the instruments used in the process	<p>Specific manufacturer and model number should be listed.</p> <p>Critical specifications should be provided (such as the g-force produced by a specific centrifuge).</p> <p>Equipment listing is meant to allow current and future researchers to duplicate the process on alternate instruments if required.</p> <p>Instrument size, such as a fixed volume pipette, should also be provided to ensure accuracy can be duplicated when the procedure is done.</p> <p>When using automated equipment, such as a nucleic acid extraction or liquid handling unit, this level of detail becomes even more critical.</p>
Procedure	Step-by-step description of the tasks involved in the process	<p>The detail should be sufficient that any qualified person could replicate the procedure by simply following the document.</p> <p>Biospecimen processing procedures must include information about time parameters for performing different steps of the task, and temperature specifications for those steps.</p> <p>Allows researchers to determine if the biomarkers desired for investigation are likely to have remained stable under the time and temperature conditions used in the original processing.</p>

QC checks should also be defined throughout the body of the SOP. A description of how and when the QC check is applied and who performs the QC check is best practice. Record-keeping requirements are defined in the body of the SOP, most often accompanied by controlled templates, worksheets or forms. Electronic or paper forms must be able to be completed in real-time. All record-keeping should be done as the process is performed, and not backfilled after the process is completed.

4.3. QA and QC Plans

QA/QC Plans should be forward-looking and customized for dual purposes: the intended use by the Study and the potential uses of biospecimens that will be archived for future research. The QA/QC plans should include:

- Personnel training and competency assessments.
- QC measures in place to ensure accuracy and integrity (e.g. bar-code scanning of vials, double entry of data, splitting of collections, assays to determine biospecimen integrity, data edit checks to ensure data accuracy).
- A monitoring plan that encompasses both data and laboratory inspections.
- Pilot studies to ensure processes developed will be suitable for the intended use.
- Curation of the Study data.

Table 4.2: Quality elements within QA/QC plans by process step, and examples of SOPs to control and assess those elements

Process step	Receipt of Samples	Inventory Acceptance	Storage Preservation	Retrieval & Distribution	QC & Reporting
Quality Elements	<ul style="list-style-type: none"> • Handling requirements • Shipment acceptance criteria <ul style="list-style-type: none"> ○ DGR compliance ○ Integrity/Temperature ○ Specimen/box quantity 	<ul style="list-style-type: none"> • Vial level inspections • Data Reconciliation • Inventory Management • QC of Specimens 	<ul style="list-style-type: none"> • Equipment Validation • Maintenance/calibration • Environmental monitoring • Security • Deviation reporting • Control of property • Business continuity 	<ul style="list-style-type: none"> • Controlled access • Inventory requirements • Shipper validation/QC • Regulatory Compliance <ul style="list-style-type: none"> ○ ICF ○ MTA ○ DGR compliance 	<ul style="list-style-type: none"> • Chain of custody • Sample lineage • Audity Trail • Inventory records
Example SOPs	<ul style="list-style-type: none"> • SOP25205: Processing Incoming Frozen Specimens • SOP25100: Receiving Fresh Blood Samples • SOP25423: Digital Documentation • SOP15110: Accessing Freezers/Refrigerators and Maintaining Specimen Temperature 	<ul style="list-style-type: none"> • SOP25205: Processing Incoming Frozen Specimens • SOP25423: Digital Documentation • SOP25104: Data Entry • SOP19087: DNA Quantitation Using the SPECTRAMax Plate Reader • SOP25105: Cell Counting and Viability Determination • SOP21069: Epstein Barr Virus Transformation of PBMC 	<ul style="list-style-type: none"> • SOP27003: Freezer Validations • SOP25213: Monitoring and Maintenance of Repository Temperature Controlled Equipment • SOP25200: Central Monitoring System • SOP25210: Procedure for Freezer Failure • SOP754.01: Control of Customer Property • SOP712.01: Business Continuity, Disaster Response & Recovery Plan 	<ul style="list-style-type: none"> • SOP25306: Data Entry Procedures for Processing Requisitions • SOP25204: Lab Processing for Sample Requisitions • SOP00108: Ordering, Receiving, Packaging and Transportation of Biologicals • SOP524.01: Repository Management Plan • SOP25207: Aliquoting of Frozen Body Fluids (Excluding DNA) • SOP19092: Aliquoting of DNA 	<ul style="list-style-type: none"> • SOP524.01: Repository Management Plan • SOP25372: NHLBI Project Specific Procedure • SOP25396: NHLBI QMS • BSI User Manual • Standard and NHLBI Specific reports

4.3.1. Training and Competency

Training indicates that a person understands a process, whereas competency measures the ability of the person to perform that process correctly. Training should be conducted and documented for all new or revised SOPs.

Methods for competency assessments can vary and may be based on observation or on performance of specific tasks with known expected outcomes such as correct entry of data or performing an assay with an expected result.

4.3.2. Quality Control

Data and biospecimen quality control measures should be put into place to ensure the accuracy of the data collected and the quality and consistency of biospecimen collections.

Examples of common quality control measures for data include:

- Pre-defined formats for all data fields
- Edit checks to confirm valid formats/values
- Double entry of hand-keyed data
- Barcode scanning of vials

Examples of common biospecimen QC measures include:

- Quantification of cell and nucleic acid biospecimens
- Purity and integrity assessment of sample derivatives
- Electronic or double-check of critical process steps
- Confirmation of biospecimen locations
- Insertion of QC samples in process stream and/or assays
- Periodic reconciliation between data sets during the active phase of the Study to ensure consistency



Figure 13: Quality inspection

4.3.3. Quality Monitoring

QA/QC Plans must include a monitoring plan that describes how quality monitoring will be performed, reported and reviewed. The Plan should include monitoring frequency,, personnel who will be doing the monitoring, the reporting schedule, and personnel who will receive and review the monitoring reports. Monitoring should include an assessment of data accuracy and biospecimen collection, processing and storage accuracy.

The aim of a quality monitoring plan is to ensure that the systems and procedures in place are effective and that staff are adhering to them. There should be a feedback mechanism in place to ensure proper corrective and preventive actions are put into place and to inform staff performing the work of any issues found.

4.3.4. Pilot Studies

QA/QC Plans frequently include pilot studies to demonstrate that the fully developed Study procedures are effective and that they are capable of providing biospecimens suitable for the research purposes they are intended to fulfill.

5. Study Documents

5.1. Overview

Biospecimens entering the Biorepository are intended for future research where the exact parameters for testing are not known. Therefore, meticulous record-keeping is essential to ensure that all relevant variables are captured. The detailed vial level data that are needed to make a biospecimen usable in the future are often in excess of the usual requirements of a standard testing lab. However, with proper planning, the annotation requirements can be easily incorporated into Study Procedures governing the trial and testing labs.

Study documentation designed to address the goal of creating a robust biospecimen collection for future scientific use should include Standard Operating Procedures (SOPs) that document the purpose, organization, methods, and procedures to be employed during the execution of the Study. Study procedures must incorporate these documentation practices to maximize the scientific utility of the biospecimens and data. Without appropriate and thoughtful resource planning, the scientific value of the biospecimens and their accompanying data sets may be diminished.

5.2. Document Library

Regardless of the future disposition of the biospecimen collection, whether it will be maintained by the original Study investigating team or transferred to a centralized biorepository, it is vital to assemble all of the key Study documentation into a document library. These documents include:

- Study protocol.
- Manual of Operations and any additional documentation including SOPs related to biospecimen collection, processing, storage, and transfer.
- Study and biospecimen worksheets and forms
- Informed consent templates and revision history.
- Laboratory test methodology, analysis, and results including reference ranges.
- Data dictionaries that define each field in the database.

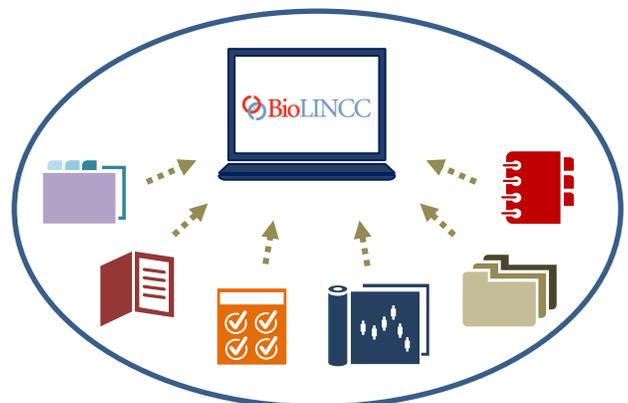


Figure 14: Building a Study document library for future use

The documentation must be sufficiently complete such that a person responsible for the use and selection of biospecimens can determine:

- How the Study was conducted.
- How the biospecimens were prepared and stored.
- How associated data was collected.

The documents must be assembled in such a way, and the details must be sufficiently in-depth, as to allow these determinations to be made without consultation with any member of the original Study team. Incomplete documentation can lead to loss of scientific utility of otherwise valuable and irreplaceable biospecimen resources.

Appendix 1

Additional Resources on Building Repository Collections

Overall Guidance	Best Practices for Repositories: Collection, Storage, Retrieval and Distribution of Biological Materials for Research	https://www.isber.org/page/BPR
	NCI Best Practices for Biospecimen Resources	https://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf
Quality Standards	FDA Regulations Relating to Good Clinical Practice and Clinical Trials	https://www.fda.gov/science-research/clinical-trials-and-human-subject-protection/regulations-good-clinical-practice-and-clinical-trials#FDARegulations
	International Organization of Standards ISO9001:2008	https://www.iso.org/iso-9001-quality-management.html
	College of American Pathologists, Biorepository Accreditation Program	https://www.cap.org/
Ethical and Legal	OHRP Policies and Guidance for Informed Consent	https://www.hhs.gov/ohrp/regulations-and-policy/guidance/informed-consent/
	Standards for Privacy of Individually Identifiable Health Information for Covered entities	https://www.hhs.gov/hipaa/for-professionals/security/index.html
	Certificate of Confidentiality for some NIH funded studies	https://grants.nih.gov/policy/humansubjects.htm
	Material Transfer Agreements	https://www.ott.nih.gov/resources#MTACTA , https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/about.html
	Research Material Distribution Agreements	https://biolincc.nhlbi.nih.gov/media/BioLINC.C.RMDA.V02.d20120806.pdf
Specimen Collection, Processing and Storage	Clinical and Laboratory Standards Institute Guidance Documents (e.g., H3-A6, H18-A4, MM13-A)	https://clsi.org/
	Assay manufacture guidelines and literature searches	
	Example Standard Operating Procedures	http://www.ctrnet.ca/resources/operating-procedures https://biospecimens.cancer.gov/resources/sops/