Creating a Biospecimen Collection for the AgingResearchBiobank

When creating a study collection, considerations must be made for the specimen types being collected, tubes used for storage, label design, data collected, and records for each specimen. It also requires careful planning of facilities' equipment and software to manage the biospecimens' inventory system.

1. Specimen (Material) Types and Volumes

1.1 Material Type

The types of biospecimens collected will depend on the downstream assays to be performed. These assays therefore determine many of the specimen collection data and storage parameters. For example, if the purpose of collecting the specimen is for testing using a Polymerase Chain Reaction (PCR) assay for example, 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, and Manual of Operations, should include procedures to collect sufficient material quantities and to process and store those materials appropriately for both planned and foreseeable future uses.

1.2 Material Volumes

Single-use frozen aliquots are what should be shared with the Biobank. These should be created at the point of sample processing to minimize the need for freeze-thaw cycles downstream. 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. Although tolerance for freeze-thaw events is assay, material type, and tissue-type dependent, thaws may introduce undesirable modifications or may damage specimen integrity by degrading critical molecules such as RNA proteins, or even minor DNA damage, and can affect some assay results (e.g., methylation state or PCR). The number and size of aliquots must balance the need for pristine biospecimens with the practical considerations of freezer space and storage costs.

An aliquot scheme should be developed for each visit and material type. The biobank typically recommends a scheme of 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.

The table below contains the most common single use aliquot sizes and aliquot schemes for various whole blood derivatives.

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

Aliquot schemes can vary widely based on the diversity and volume of materials to be collected. 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. Study groups anticipating submitting biospecimens should consult the biobank prior to finalizing their aliquot scheme, to determine the number and size of aliquots that will be prepared specifically for future use and submitted to the Biobank.

2. Vial/Tube Selection

The tube that will be used to hold the material must be specifically designed and tested to withstand ultra-cold units such as -80°C freezers and liquid nitrogen freezers (-180°C). Tube size should be selected based on the material volumes being stored. For example, 0.2 ml of material would be recommended to be stored in a 0.5mL tube vs a 2.0 mL tube. It is also recommended that tubes are externally threaded to avoid overfilling.

3. 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 must maintain the link to the Study participant data. Non-barcoded labels, labels with handwritten information, and labels with private participant information are generally not accepted for transfer to the Biobank. In addition, each vial must have a coded identifier that is unique at the vial level. (i.e., aliquots of one sample or blood draw cannot share exactly the same identifier, but must have at a minimum an additional aliquot number that differentiates each vial from its sister aliquots. Vials without unique identifiers require additional resources to inventory, retrieve, and distribute and are only accepted into the Biobank if the collection is unique and

has significant potential scientific value, and if resources are available to support the additional level of effort required to manage them.

3.1 Label Stock

The physical labels applied to vials or containers containing biospecimens must be tested to ensure that labels applied will remain readable and adherent to the vials/ containers throughout their 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.

Each vial should have a single label, and the label should fit on the vial without obscuring the vial contents. All information must be printed in permanent ink (as mentioned above, no handwritten information). The label should be applied to the vial with the barcode in a vertical orientation to allow for scanning, and the label should not overlap, leaving a gap available to visualize the volume.

3.2 Label Information

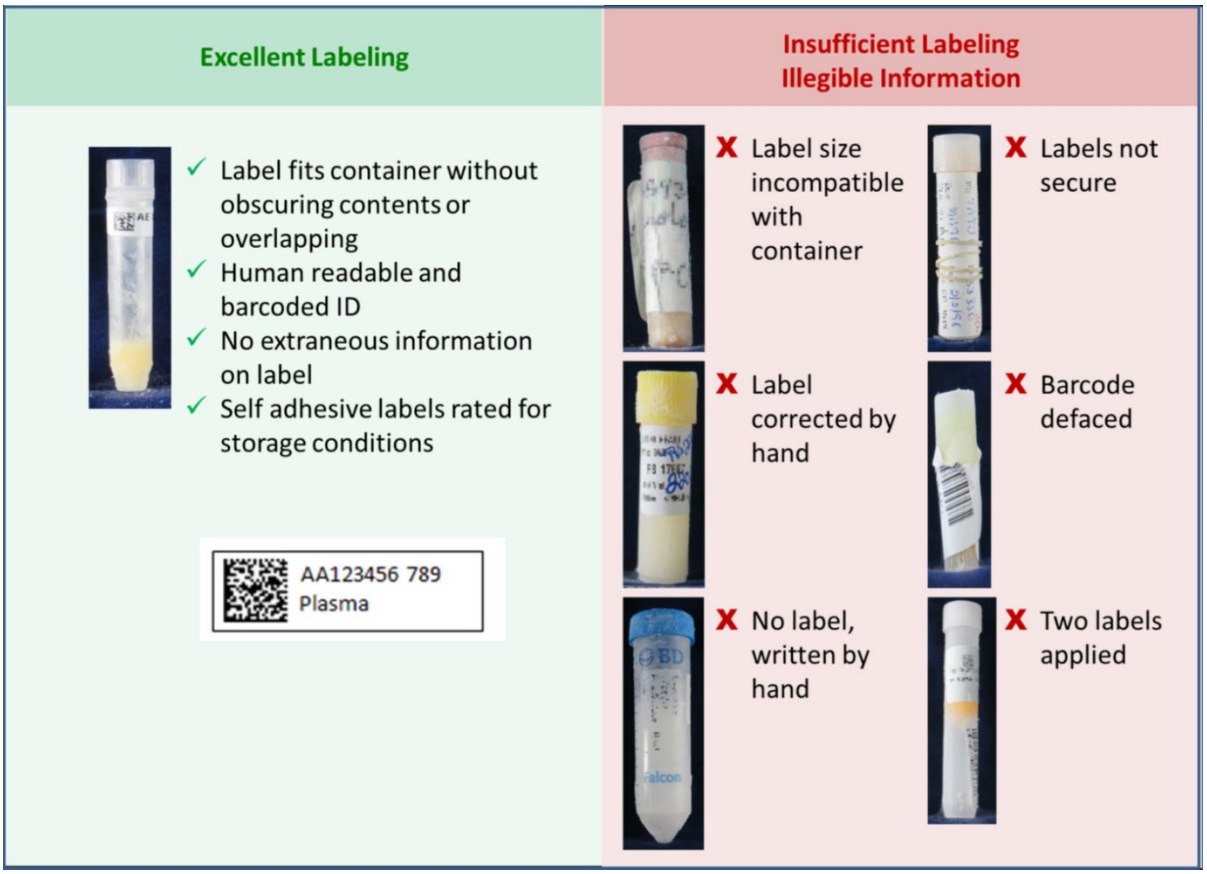
The information on the label should be simple and follow the same configuration for all vials in the study. The information should include the following:

- Unique Vial Identifier Barcode: Unique identifier assigned to a vial
- Eye-Readable Vial Identifier: Alphanumeric representation of the barcode noted above
- Material Type: The type of material collected (e.g. serum, plasma, whole blood, urine, DNA, etc.)

If the Study's IRB requires a different deidentified ID to be used in the accompanying clinical data to maintain patient privacy, vials whose labels include the original Study Participant IDs may need to be relabeled by the Study before deposition in the Biobank. For this reason, it is not advised to include the Study's Participant ID on vial labels.

Labels and barcodes must be examined prior to applying them to samples. This should include a visual check to ensure the quality of the printed information to minimize formatting issues such as the print being too small, illegible font/font cut off from the edge of the label, dark/splotchy printing, faded printing, etc. It is critical to confirm that there is sufficient "white space" surrounding the barcode to ensure its ability to be scanned. The barcode should be tested to ensure it can be scanned reliably by all parties that will be handling the specimens.

Below is an example of a well-labeled vial and barcode vs. examples of insufficient or illegible labeling.



4. Consent

Study participants who donate biospecimens must have been 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. Biospecimens that are not consented for future research by non-Study investigators, or which have a finite retention period, may not be accepted into the Biobank.

Collections transferred to the biobank must have an electronic data file that links each specimen to the informed consent data regarding its current or 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 must 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 NIA/AgingResearchBiobank, and the potential for reduction of the collection if not used.

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

The AgingResearchBiobank also has relevant information to help investigators develop management and sharing plans for their data:
https://agingresearchbiobank.nia.nih.gov/investigator-help/

5. Specimen storage

Specimens should be stored in appropriate box sizes that were validated and tested for ultra-cold storage. Vials should be placed in boxes with tight-fitting lids and no space should be left to ensure a vial does not become dislodged during handling and transit.

There must be adequate facilities to store and maintain the collection during the life of the Study. Adequate and appropriate freezer space will be required to house the collection. In addition, equipment and qualified 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.

5.1 Biospecimen Inventory System

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 system must be 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”) and linking it to 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.

6. Quality Control

Data and biospecimen Quality Control (QC) measures should be put into place to ensure the accuracy of the data collected and the quality and consistency of biospecimen collections throughout the life of the collection.

Examples of common QC 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 vial labels

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 QC of sample locations compared to biospecimen inventory data
- Periodic reconciliation between data sets during the active phase of the Study to ensure consistency

6.1 Quality Monitoring

Quality Assurance (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 accuracy of storage locations.

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.

7. Pilot Studies

The biobank recommends including in the QA/QC Plans a pilot study 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.

8. Required Data and Documentation for Submission to the Biobank

The following must be submitted to the NIA/AgingResearchBiobank for approval prior to acceptance of the collection:

- Example of sample labels
- Example of shipping manifest
- Copy of the Informed Consent Template(s) used by the study
- Copy of the study protocol and Manual of Operation (MOP)
- Brief description of Study Investigator procedures to collect, track and monitor biospecimen processes – from collection through shipping to the NIA/AgingResearchBiobank
- Specific secondary uses that the collected data and/or biospecimens are suitable for and how the collection will continue to contribute to the field of Aging Research

Upon acceptance of the collection, the following will be required:

- Full biospecimen inventory, including vial locations
- A link between the biospecimen IDs and the participant IDs used in the clinical data that are associated with the biospecimens