Risk Factors for Future Cognitive Decline and Alzheimer's Disease in Older African Americans (Pathways) Study

in collaboration with the

National Centralized Repository for Alzheimer's Disease and Related Dementias

Biospecimen Collection, Processing, and Shipment Manual of Procedures

Version April 2025

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1.0 ABBREVIATIONS

AD	Alzheimer's Disease
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
NACC	National Alzheimer's Coordinating Center
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PATH	Risk Factors for Future Cognitive Decline and Alzheimer's Disease in Older African Americans Study (Pathways Study)
PHI	Protected Health Information
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

2.0 PURPOSE

The collection of biofluids is an important part of the Pathways Study. The purpose of this manual is to provide study staff with instructions for collection and submission of biological samples for Pathways study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

The following samples will be sent to NCRAD:

- Plasma
- Whole blood (DNA Extraction)

This manual includes instructions for collection of blood, fractionation of blood from collection tubes, aliquoting, labeling, storage prior to shipping, and shipping to NCRAD.

These procedures are relevant to all study personnel responsible for processing specimens provided to NCRAD for the Pathways protocol.

3.0 NCRAD Information

3.1 NCRAD Contacts

Tatiana Foroud, PhD, Core Leader
Phone: 317-274-2218

Kelley Faber, MS, CCRC, Project Manager
Phone: 317-274-7360
Email: kelfaber@iu.edu

Abigail Erickson, BS, CCRP, Clinical Research Coordinator
Phone: 317-278-1133
Email: agericks@iu.edu

General NCRAD Contact Information
Phone: 1-800-526-2839 or 317-278-8413
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: www.ncrad.org

Sample Shipment Mailing Address
Pathways at NCRAD
Indiana University School of Medicine
351 W. 10th St. TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839 or 317-278-8413

3.2 NCRAD Hours of Operation

Indiana University business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Frozen samples must be shipped **Monday-Wednesday only.**

For packing and shipment details of samples, please refer to Section 8.0 of this protocol.

Check the weather report to make sure impending weather events (blizzards, hurricanes, etc.) will not impact the shipping or delivery of the samples.

3.3 NCRAD Holiday Observations

Date	Holiday
January 1	New Year's Day
3rd Monday in January	Martin Luther King, Jr Day
4th Monday in May	Memorial Day
June 19	Juneteenth
July 4	Independence Day
1st Monday in September	Labor Day
4th Thursday in November	Thanksgiving
4th Friday in November	Friday after Thanksgiving
Winter Break	Christmas Day through New Year's Day

Please note that between December 24th and January 2nd, Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2nd. If possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University after the second week in December. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 20th by e-mailing alzstudy@iu.edu, so that they can arrange to have staff available to process incoming samples. **Please see: https://ncrad.org/holiday_closures.html for additional information.**

- Please note that courier services may observe a different set of holidays.
- Please be sure to verify shipping dates with your courier prior to any holiday.
- **Weekend/holiday delivery must be arranged in advance with NCRAD staff.**

4.0 PATHWAYS LABORATORY COLLECTION

4.1 Site Required Equipment

The following materials and equipment are necessary for the processing of specimens at the collection site and are to be **supplied by the local site**:

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquet
- Alcohol Prep Pad
- Gauze Pad
- Bandage
- Butterfly needles and hub
- Microcentrifuge tube rack
- Sharps bin and lid
- Pelleted dry ice

In order to process samples consistently across all projects and ensure the highest quality samples possible, project site must have access to the following equipment:

- Centrifuge capable of ≥ 2000 x g
- -80°C Freezer

In order to ship specimens, you must provide:

- Pelleted dry ice (approximately 45 lbs per shipment)

4.2 Biospecimens Sent to NCRAD

Samples are to be submitted according to the shipping methods outlined in Section 8.0. Guidelines for the processing, storage location, and timing of sample collection are listed in the tables below.

5.2.1 Biofluid Collection Schedule

Biospecimen Collection Table

	Every Participant Visit (AA, AAL, COV) 1-7 & E
Plasma	X
Whole Blood (for DNA extraction)	X

Whole blood is collected in four collection tubes (3 x 10 ml purple-top EDTA tubes and 1 x 6 ml purple-top EDTA tube) for shipment to NCRAD. The 10 ml EDTA tubes are processed locally into plasma fractions; they are then aliquoted, frozen at the study site, and shipped to NCRAD. The 6 ml purple-top EDTA tube is shipped frozen to NCRAD without any processing at the site.

Consent forms must specify that any biological samples and de-identified clinical data may be shared with academic and/or industry collaborators through NCRAD. Recommended consent language can be found on the NCRAD website at: https://ncrad.org/recommended_consent_language.html. A copy of the consent form for each participant should be kept on file by the site investigator.

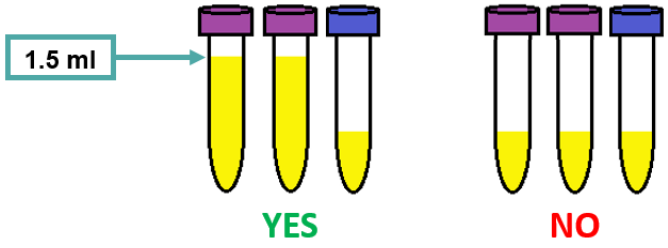
4.2.2 Biofluid Collection Charts

Collection Tube	Drawn At	Specimen Type	Aliquot Volume	Total Number of Aliquots	Shipping Temperature
3 EDTA (Purple-Top) Blood Collection Tube (10 ml)	All Visits	Plasma	1.5 ml plasma aliquots	Up to 10	Frozen
1 EDTA (Purple-Top) Blood Collection Tube (6 ml)	All Visits	Whole Blood	N/A	N/A	Frozen

4.3 Filling Cryovials

In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to the assigned volume after processing is completed (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of sample.

Aliquot the remaining biologic material as the residual volume and ship to NCRAD. Ship *all* material to NCRAD. Fill as many aliquot tubes as possible. For example, if 2.7 ml of a plasma sample is obtained, fill 1 cryovial with 1.5 ml, and one additional cryovial with the remaining 1.2 ml.



Please note: It is critical for the integrity of future studies using these samples that study staff note if an aliquot tube contains a residual volume (anything under 1.5 ml for plasma). Please highlight that the aliquot contains a small volume by utilizing the blue cryovial cap provided in each kit. Please record the last four digits of the residual aliquot on the Biological Sample and Notification Form.
If there are any unused cryovials, please do not send the empty cryovials to NCRAD. These unused cryovials (ensure labels are removed) can be saved as part of a supplemental supply at your site or the cryovials can be disposed of per your site's requirements.

To assist in the preparation and aliquoting of samples, colored caps are used for the aliquot tubes. The chart below summarizes the association between cap color and type of aliquot.

Cap Color	Sample Type
Lavender	Plasma
Blue	Residual sample plasma

5.0 Specimen Collection Kits, Shipping Kits, and Supplies

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD, the Blood Supplemental Supply Kit, the Frozen Shipment Kit and 2) clinical lab supplies (with the exception of dry ice and equipment supplies listed in Section 5.1). The provided materials include blood tubes, pipettes, boxes for plasma aliquots, as well as partially completed

shipping labels to send materials to NCRAD. Kit number labels, site and PTID labels, collection tube labels, and aliquot labels will all be provided by NCRAD. Details regarding the blood kits are found in this Manual of Procedures. Collection tube and aliquot labels will be preprinted with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 7.1.

5.1 NCRAD Specimen Collection Kit Contents

Collection kits contain the following (for each participant) and provide the necessary supplies to collect samples from a given participant. Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have received approval from the NCRAD Study team to do so. *Please store all kits at room temperature until use.*

Blood Kit

Quantity	Blood Kit Components
3	EDTA (purple-top) blood collection tube (10 ml)
1	EDTA (purple-top) blood collection tube (6 ml)
9	Cryovial (2.0 ml) with purple cap
1	Cryovial (2.0 ml) with blue cap
1	50 ml conical
1	Bubble wrap tube sleeve
14	Preprinted Collection Tube and Aliquot Labels (3x WBLD EDTA10; 10x PLA; and 1x WBLD EDTA6)
3	Preprinted Kit Number Label (1 for sample form, 1 for cryobox, 1 extra)
5	Label for handwritten Sequence ID (3 for 10ml EDTAs, 1 for 6ml EDTA, and 1 extra)
1	Disposable graduated transfer pipettes (3 ml)
1	Resealable Bag
1	Resealable small poly bag (4” x 6”)

Supplemental Supply Kit

Quantity	PATH Supplemental Supply Kit Components
9	EDTA (purple-top) blood collection tube (10 ml)
3	EDTA (purple-top) blood collection tube (6 ml)
27	Cryovial (2.0 ml) with purple cap
3	Cryovial (2.0 ml) with blue cap
12	Label for handwritten Sequence ID
2	25 cell Cryovial box
3	Disposable graduated transfer pipettes (3 ml)
3	Unwrapped 50ml conical
3	Bubble wrap tube sleeve
1	Resealable bag
1	Kit bag label

NCRAD Frozen Shipping Supply Kit

Quantity	Frozen Shipping Kit Components for Blood-Based Biomarkers
8	Plastic Biohazard bag with absorbent sheet (small)
8	25 cell Cryovial box (put samples from 2 participants per cryobox)
1	Shipping pouch
1	Shipping box/Styrofoam container
1	Un3373 Label
1	Fragile Label
1	Dry Ice Label
1	Resealable bag

Individual Supplies

Quantity	Items Available upon request within the NCRAD kit module
By Request	Cryovial box (holds up to 25 cryovials)
By Request	Cryovial (2.0 ml) with purple cap
By Request	Cryovial (2.0 ml) with blue cap
By Request	Shipping container for dry ice shipment (shipping and Styrofoam box)
By Request	Styrofoam shipping containers (11”x 9”x 8”, 1 1/2” wall)
By Request	Plastic biohazard bag with absorbent sheet (small)
By Request	Disposable graduated transfer pipette (3 ml)
By Request	EDTA (Purple-Top) Blood Collection Tube (10 ml)
By Request	EDTA (Purple-Top) Blood Collection Tube (6 ml)
By Request	50 ml conical
By Request	Bubble wrap tube sleeve
By Request	UN3373 label (category B label)
By Request	Fragile label
By Request	Dry ice shipping label
By Request	Shipping pouch
By Request	Fine Point Permanent Markers
By Request	Sequence ID Labels

5.2 Kit Supply to Study Site

Each site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits. Please go to: https://redcap.uits.iu.edu/surveys/?s=AJYP4X7MMKRNWTD3 to request additional kits and follow the prompts to request the desired supplies.

Please allow **THREE weeks** for kit orders to be processed and delivered.

6.0 Blood Collection and Processing Procedures

6.1 Labeling Samples

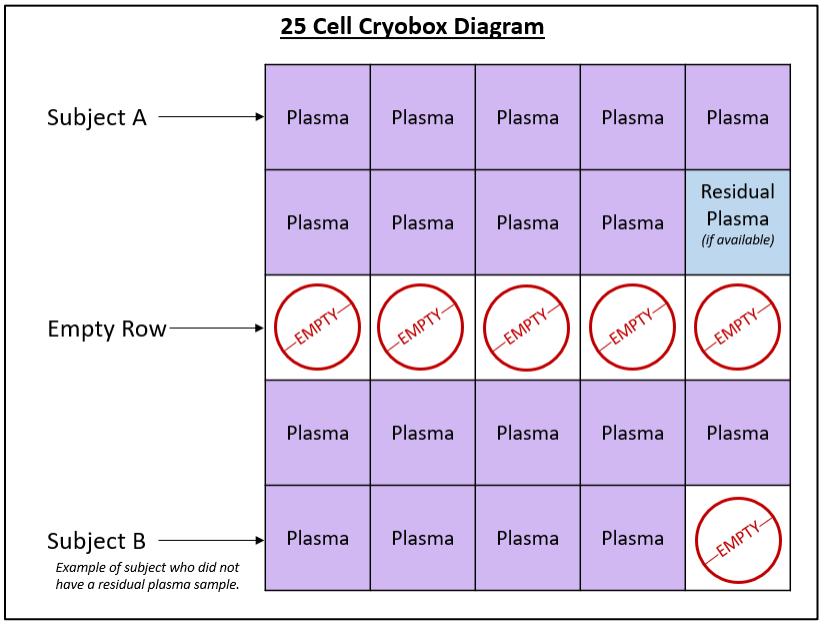
Important Note: In order to ensure the highest quality samples are collected, it is essential to follow the specific collection and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood.

Label Type Summary:

1. Kit Number Label
2. Sequence ID Label
3. Collection and Aliquot Tube Label

Kit Number 1000001	**Kit Number Labels** tie together all specimens collected from one participant at one visit. They should be placed on each cryobox, and in the designated location on the Blood Shipment Notification Forms.
Sequence ID PATH _____	**Sequence ID Labels** are used to document the individual's unique study ID. Place labels on the blood collection tubes (3 x EDTA 10 ml Tube and 1 x EDTA 6ml Tube).
PATH COLLECT Kit: 1000002 1000000124 WBLD EDTA10 PATH COLLECT Kit: 1000002 1000000124 WBLD EDTA6	Place one **Collection Tube Label** on each blood collection tube. These labels are specimen-specific. They will be labeled with: WBLD & EDTA10 *or* WBLD & EDTA6
PATH ALIQUOT Kit: 1000002 1000000124 PLA EDTA10	Place one **Aliquot Tube Label** on each cryovial. These labels are specimen-specific.

Each cryobox will contain samples from 2 different participant visits: Samples should be arranged according to the diagram below. An individual participant's samples should be placed within two rows. An empty row should separate one participant's samples from another participant's samples. Full 1.5ml plasma aliquots should be placed into the first available cells, with the residual sample immediately to the right of the last 1.5ml plasma aliquot.



Because each cryobox will contain samples from 2 different participant visits**, the top of the cryobox will end up being labeled with 2 different kit number labels**, each corresponding to the samples within the box.

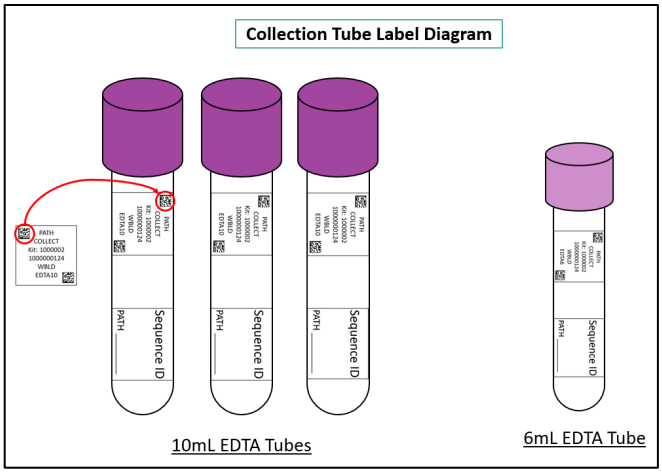
Photo outlining how to fit 2 Kit Number Labels onto one cryobox lid.

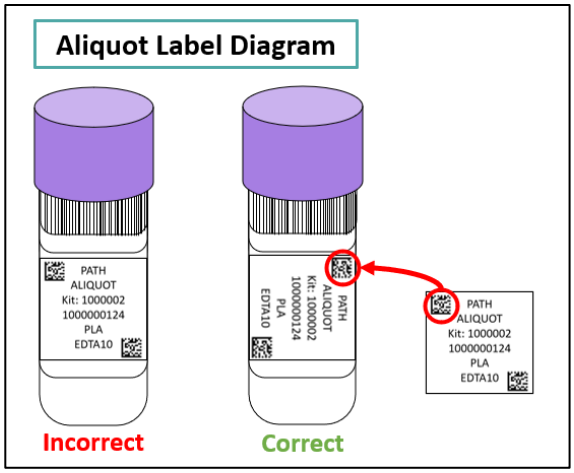
The 10mL and 6mL EDTA (Purple-Top) collection tubes will have two labels: the Collection Tube Label and the Sequence ID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten Site and PTID label toward the bottom.

In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place cryovial labels on **ALL** cryovials **BEFORE** sample collection. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Using a fine point permanent marker, fill-in and place the Sequence ID labels on the EDTA (purple-top) tubes **BEFORE** sample collection. These labels are placed on collection tubes in addition to the collection tube label.
- The collection tube labels contain a 2D barcode on the left-hand side of the label. Place this barcode toward the tube cap.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright).

Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label.





For a blood collection visit, tubes should be labeled according to the chart below.

Kit Number
1000001

3 x Kit Number Labels
- Place one on the Sample Form.
- Place the other on the outside of the cryobox holding the samples
- There will also be one extra label

Sequence ID
PATH _____

5 x Sequence ID Label
- Place on the 3 x 10mL EDTA purple-top blood collection tubes and the 1 x 6mL EDTA purple-top blood collection tube
- There will also be one extra label

PATH
COLLECT
Kit: 1000002
1000000124
WBLD
EDTA10

3 x Collection and Aliquot Tube Label – WBLD EDTA10
- Place the 3 labels with specimen type WBLD and Collection Container EDTA10 on the 10mL EDTA purple-top blood collection tubes

PATH
ALIQUOT
Kit: 1000002
1000000124
PLA
EDTA10

10 x Collection and Aliquot Tube Label – PLA EDTA10
- Place these labels on the 9 on the purple-top cryovials and one blue-top cryovial

PATH
COLLECT
Kit: 1000002
1000000124
WBLD
EDTA6

1 x Collection and Aliquot Tube Label – WBLD EDTA6
- Place the label with specimen type WBLD and Collection Container EDTA6 on the 6mL EDTA purple-top blood collection tube

6.2 Whole Blood Collection with 10 ml EDTA (Purple-Top) Tubes for Plasma

1. Store empty EDTA tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use. Ensure that the tubes have not expired.

2. Place labels according to the labeling instructions in section 6.1.

3. Using a blood collection set and a holder, collect blood into the **3 x EDTA (Purple-Top) Blood Collection Tubes (10 ml)** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:
   a. Place participant's arm in a downward position.
   b. Hold tube in a vertical position, below the participant's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
   a. If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.

5. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tubes 8-10 times.

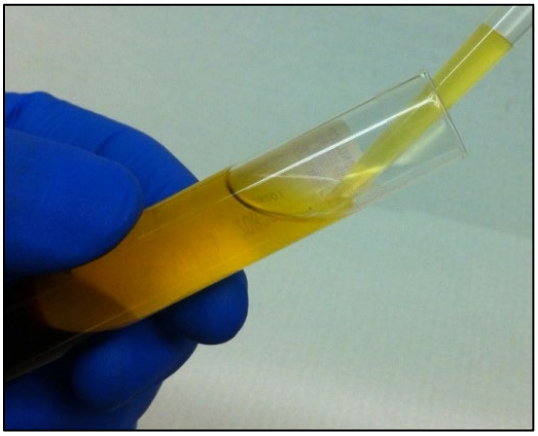
6. Sample transfer to processing lab.

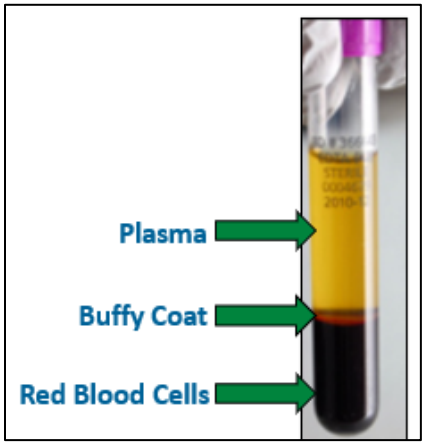
7. Centrifuge balanced tubes for 10 minutes at 2000 x g at room temperature (20°C). **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix A to calculate equivalent RPM for spin at 2000 x g).**
   a. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
   b. Record original volume drawn for each tube in spaces provided on the Biological Sample Shipment and Notification Form.
   c. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
   d. Record time aliquoted on the Biological Sample Shipment and Notification Form.

8. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall being careful not to agitate the packed red blood cells at the bottom of the collection tube.

9. Transfer plasma from all three EDTA tubes into the 50 ml conical tube and gently invert 3 times. **NOTE**: When aliquoting the plasma from the EDTA tube, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

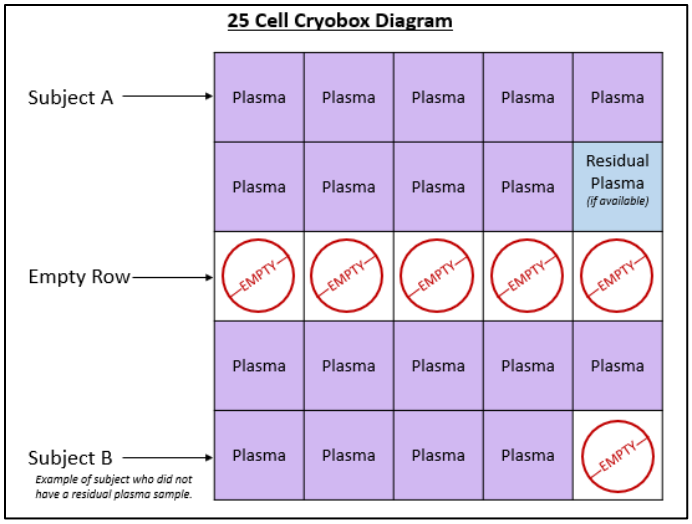
10. Aliquot 1.5 ml plasma per purple-cap cryovial. Place residual plasma (<1.5 ml) in the blue-capped cryovial. **If a residual aliquot (<1.5 ml) is created, document the specimen number and volume on the Biological Sample and Shipment Notification Form.**





11. Place the labeled cryovials in a 25 cell cryobox. Label the cryobox with a kit number label corresponding to the samples.

12. Place the filled, labeled cryobox on pelleted dry ice. **Transfer to -80°C Freezer when possible**. Completely freeze and store all samples at **-80°C until shipped** to NCRAD on pelleted dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample Shipment and Notification Form.
    a. You should place samples from 2 participant visits per 25 cell cryobox. Arrange the samples according to the diagram below. An individual participant's samples should be placed within two consecutive rows. An empty row should separate each participant's samples from another participant's samples.



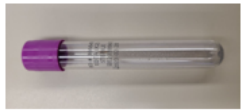
Each 25 cell cryobox should contain samples from 2 participant visits.
Each participant visit will have up to 10 x Plasma Aliquots.

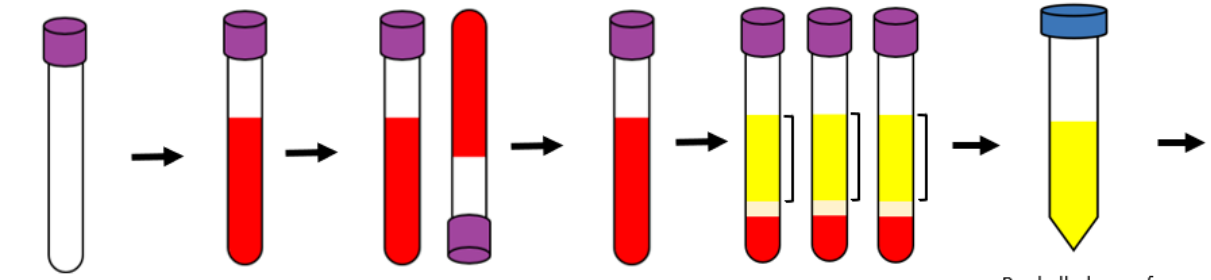
13. Dispose of collection tubes with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.

Note: A minimum of 3mL of plasma is needed per participant visit in order to receive free biomarker assays. If less than 3mL of plasma is obtained, please still send the samples to NCRAD, just know that free biomarker analysis will not be performed.

Plasma Preparation EDTA Purple-Top Tubes (3 x 10 ml)

Plasma Preparation (10mL EDTA Tube x 3)





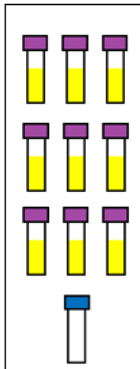
- Store tubes at room temp
- Each tube should be labeled with Collection Tube and PTID labels.

- Collect Blood into 3 EDTA tubes, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

- Immediately after blood draw, invert tubes 8-10 times to mix samples.

- Centrifuge sample at 2000 x g for 10 minutes at room temperature (20°C).

- Pool all plasma from the 3 EDTTA tubes into a 50mL conical tube and invert gently 3 times to mix the plasma.



- Label purple-capped cryovials with "PLASMA" labels.
- Using a transfer pipette Aliquot 1.5 ml of plasma into each cryovial.
- If residual aliquot is created, use the blue-capped cryovial and a "PLASMA" label. Document specimen number and volume on Sample Form
- Store plasma aliquots upright at -80°C until shipment to NCRAD

Ensure tubes are not expired prior to blood draw

Spin, aliquot, and freeze all plasma aliquots within 2 hours of collection

Please be sure to compare the labels on each tube and cryovials to the Biological Sample Form included with each kit

6.3 Whole Blood Collection with 6mL EDTA (Purple-Top) Tube

1. Store empty EDTA tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use. Ensure that the tubes have not expired.
2. Place labels according to the labeling instructions in section 6.1.
3. Using a blood collection set and a holder, collect blood into the **1 x EDTA (Purple-Top) Blood Collection Tubes (6 ml)** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:
   a. Place participant's arm in a downward position.
   b. Hold tube in a vertical position, below the participant's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 6 ml of blood into the tube.

   a. If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.

5. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tubes 8-10 times.
6. Transfer the tube immediately to a -80°C Freezer. The sample should be frozen and stored UPRIGHT in a WIRE or PLASTIC type test tube rack (DO NOT use a solid Styrofoam test tube holder).
7. Store sample at **-80°C until shipped** to NCRAD on pelleted dry ice.

Whole Blood Collection (1 x 6ml EDTA Purple Top Tube)

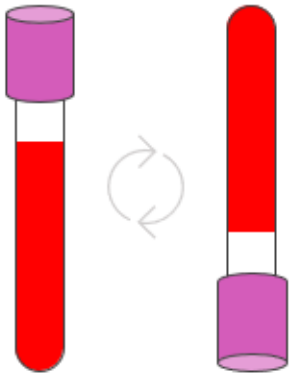
Step 1

- Store tubes at room temperature.
- Labels tubes with pre-printed subject labels prior to blood draw.

Step 2

- Collect blood in EDTA Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step 3



- Immediately after blood draw, invert tube 8-10 times to mix samples.

Step 4

- Immediately after inversion, freeze the sample in a -80°C freezer until shipment.

Ensure tubes are not expired prior to blood draw

Please be sure to compare the labels on each tube and cryovials to the Biological Sample Form included with each kit

7.0 PACKAGING & SHIPPING INSTRUCTIONS

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If you have difficulty finding biospecimen shipping training, please notify a NCRAD coordinator.

In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by NCRAD for each sample type. Investigators and clinical coordinators for each project are responsible to ensure the requested amounts of each fluid are collected to the best of their ability and that frozen samples are packed with sufficient amounts of pelleted dry ice to avoid thawing in the shipment process.

7.1 Frozen Packaging Instructions

The most important issue for shipping is to maintain the temperature of the samples. The frozen samples must never thaw; not even the outside of the tubes should be allowed to defrost. This is best accomplished by making sure the Styrofoam container is filled completely with **pelleted** dry ice.

Important Note: FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY!

Specimens being shipped to NCRAD should be considered as Category B UN3373 specimens and as such must be tripled packaged and compliant with IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

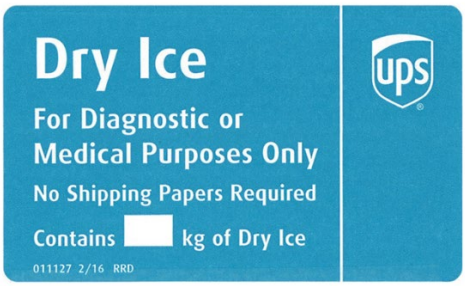
Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

***** Packing and Labeling Guidelines *****

- The primary receptacle (cryovial) must be leak proof and must not contain more than 1L total.
- The secondary packaging (biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples

of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.

- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
    - Sender's name and address
    - Recipient's name and address
    - Responsible Person
    - The words "Biological Substance, Category B"
    - UN3373
    - UPS Dry Ice label and net weight of dry ice contained

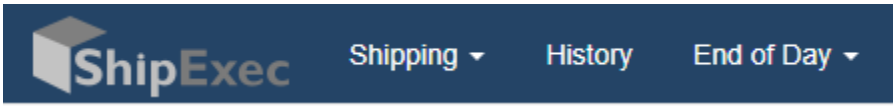


7.2 Frozen Shipping Instructions

1. Shipping samples to NCRAD should be instigated when samples from 16 participant visits have been collected. Each frozen batch shipment to NCRAD can hold samples from up to 16 participant visits (8 x cryoboxes with samples from up to 2 participant visits per cryobox.

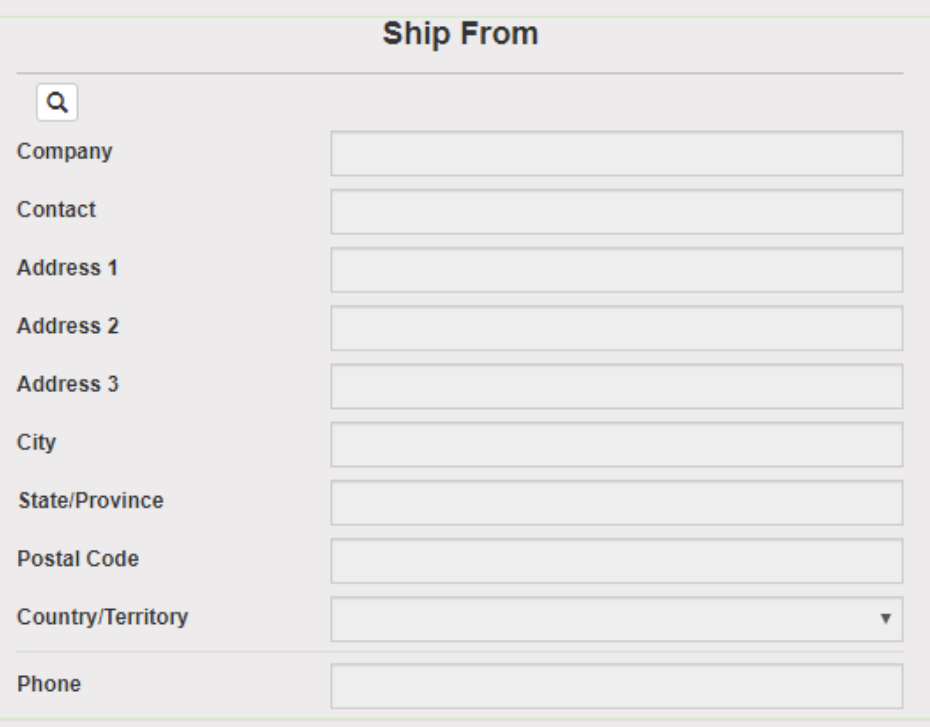
2. Log into the ShipExec Thin Client at: kits.iu.edu/UPS.
    a. If a new user or contact needs access, please reach out to your study contact for access.

3. Click "Shipping" at the top of the page and select "Shipping and Rating".



4. Select your study from the "Study Group" drop down on the right side of the main screen (PATH). Choosing your study will automatically filter the address book to only addresses within this study.

5. Click on the magnifying glass icon in the "Ship From" section to search for your shipping address.



   a. Search by Company (site), Contact (name), or Address 1 (first line of your site's street address). Click **Search**.
   b. Click **Select** to the left of the correct contact information.
6. Verify that both the shipping information AND study reference are correct for this shipment.
   a. If wrong study contact or study reference, click **Reset** in the bottom right of the screen to research for the correct information.

7. Enter Package Information
   a. Frozen shipments
      i. Enter the total weight of your package in the "Weight" field.
      ii. Enter the pelleted dry ice weight in the "Dry Ice Weight" field.
      iii. If the "Dry Ice Weight" field is higher than the "Weight" field, you will receive an error message after clicking Ship and need to reenter these values.
   b. Click **Ship** in the bottom right of the page when complete.

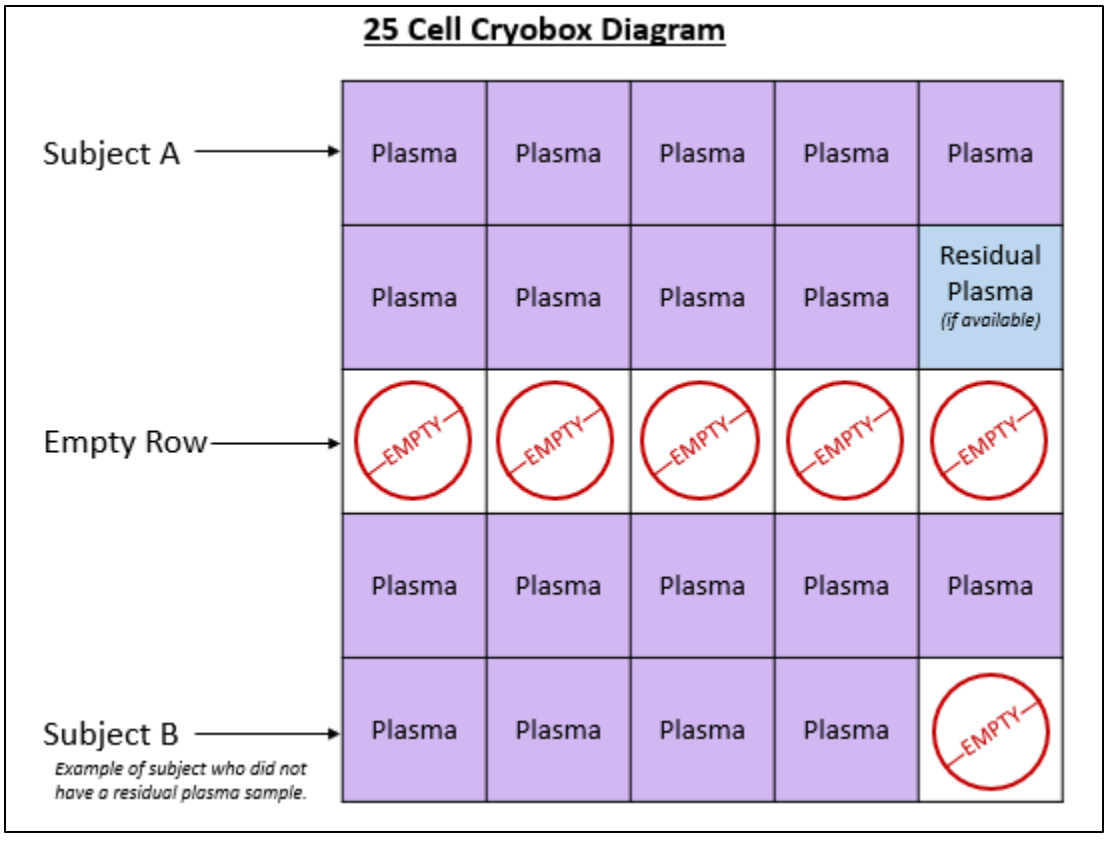
8. If your site does not already have a daily UPS pickup, you can schedule one here.
   a. Click the blue **Pickup Request** button. Enter the earliest pickup time and latest pickup time in 24-hr format.
   b. Give a name & phone number of someone who the UPS driver can call if having issues finding the package.
   c. Give the Floor and Room Number (if needed) to be as descriptive as possible where this package needs to be picked up from. Click **Save**.

9. Print the airbill that is automatically downloaded.
   a. To reprint airbill, click **History** at the top left of the page.
      i. Shipments created from the user that day will automatically populate. If shipments from a previous day need to be located, search by ship date.

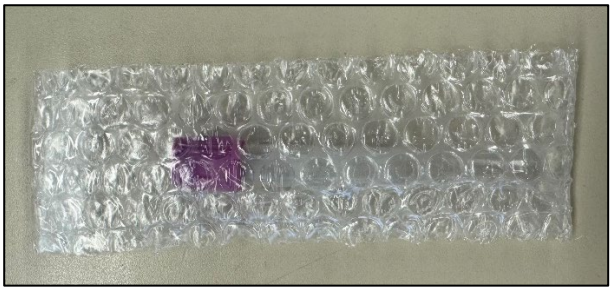
ii. Locate the correct shipment, and click on the printer icon to the left of the tracking number under "Action" to reprint the airbill
iii. Click print icon on right side of the tracking number line.

10. Fold airbill, and place inside plastic UPS sleeve. Peel the back off of the UPS sleeve and stick the sleeve to the top of the package. Do NOT overlap other labels or package seams.

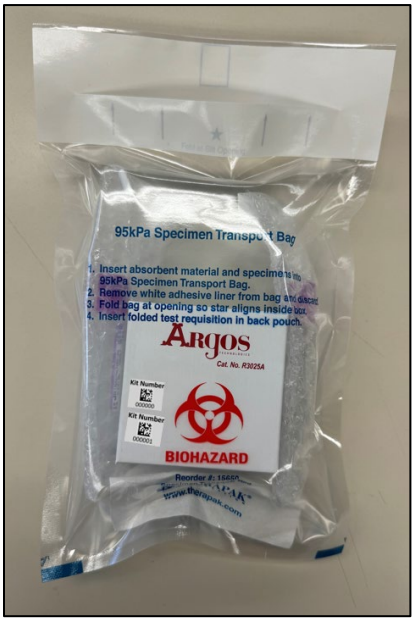
11. Notify NCRAD of shipment by emailing NCRAD coordinators at alzstudy@iu.edu. Attach the following to the email:
    a. Completed Sample Form (Appendix B) to the email notification (email NCRAD coordinator prior to shipment to receive sample form).
    b. If email is unavailable please call NCRAD at 1-800-526-2839 (or 317-278-8413) and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.
12. Cryoboxes should contain samples from 2 participant visits.
    a. Each participant visit will have up to 10 x Plasma Aliquots.
    b. This means there should be 18-20 samples per cryobox.
    c. Ensure all labeled and frozen plasma aliquots from a single participant visit are grouped within two consecutive rows in a cryovial box. Ensure that the outside of the cryovial box is labeled with the kit number label.



13. Place the frozen, filled, and labeled 6mL EDTA tube into the bubble wrap tube sleeve.



14. Place cryovial box and the 2 x bubbled-wrapped 6mL EDTA tubes from the corresponding visits in a clear biohazard bag.



15. Note: As the samples are placed in the plastic biohazard bag, do NOT remove the absorbent material found in the bag. Seal according to the instructions on the bag.

16. Seal the biohazard bag according to the instructions on the bag.

17. Place approximately 2-3 inches of pelleted dry ice in the bottom of the Styrofoam shipping container.

18. Place the biohazard bags into the provided Styrofoam-lined shipping container on top of the pelleted dry ice. **You can place up to 8 cryoboxes and 8 x 6ml EDTA tubes per shipper**. Please ensure that cryovial boxes are placed so the cryovials are upright in the shipping container. After the samples have been placed into the shipping container, completely fill the inner Styrofoam with pelleted dry ice pellets to ensure the frozen state of the specimens during transit.

19. Replace the lid on the Styrofoam carton. Place the completed Blood Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape.

20. Complete the UPS Dry Ice Label with the following information:
    a. Net weight of dry ice in kg (must match amount on the airbill)
    b. Do not cover any part of this label with other stickers, including preprinted address labels.

21. Apply all provided warning labels and the preprinted UPS return airbill to the outside of package, taking care not to overlap labels.

Important Note: Complete the required fields on the UPS Dry Ice label or UPS may reject or return your package.

22. If possible, hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off. If storage in a -80°C freezer until UPS pick-up is not possible, package samples no more than 4 hours before the expected pick-up time.

23. Specimens should be sent to the below address via UPS Next Day Air. Frozen shipments should be sent **Monday through Wednesday ONLY** to avoid shipping delays on Thursday or Friday.

    Pathways at NCRAD
    Indiana University School of Medicine
    351 W. 10th St. TK-217
    Indianapolis, IN 46202

24. Use UPS tracking to ensure the delivery occurs as scheduled and is received by NCRAD. Please notify NCRAD by email (alzstudy@iu.edu) that a shipment has been sent and include the UPS tracking number in your email.

8.0 Data Queries and Reconciliation

Sample and Shipment Notification forms must be completed on the day that samples are collected (for ambient samples), or before sample shipment (for batch frozen samples) because they include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

NCRAD will collaborate with the data team at Rutgers to reconcile information captured in the Rutgers database compared to samples received and logged at NCRAD. Additional discrepancies may be sent directly to the site staff to reconcile.

Data queries or discrepancies with samples shipped and received at NCRAD may result from:

- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Blood Sample and Shipment Notification Form and logged at NCRAD compared to information entered into the NACC database.

9.0 Appendices

Appendix A: Rate of Centrifuge Worksheet

Please complete and return this form by fax or email to the NCRAD Project Manager if you have any questions regarding sample processing. The correct RPM will be sent back to you.

Submitter Information

Name: **Site:**

Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

Determine the centrifuge's radius of rotation (in mm) by measuring distance from the center of the centrifuge spindle to the bottom of the device when inserted into the rotor (if measuring a swing bucket rotor, measure to the middle of the bucket).

Calculating RPM from G-Force:

$$RCF = \left(\frac{RPM}{1{,}000}\right)^2 \times r \times 1.118 \quad \Rightarrow \quad RPM = \sqrt{\frac{RCF}{r \times 1.118}} \times 1{,}000$$

RCF = Relative Centrifugal Force (G-Force)
RPM = Rotational Speed (revolutions per minute)
R= Centrifugal radius in mm = distance from the center of the turning axis to the bottom of centrifuge

Comments:

Please send this form to NCRAD Study Coordinator

317-321-2003 (Fax) **alzstudy@iu.edu**

Appendix B: Blood Sample and Shipment Notification Form

Please email or fax the form on or prior to the date of shipment.

Participant Sequence ID: PATH ___ ___ ___ ___

Biological Sample and Shipment Notification Form

Please email or fax the form on or prior to the date of shipment

To: Kelley Faber | Email: alzstudy@iu.edu | Phone: 1-800-526-2839

General Information:

Kit Barcode:

Coordinator Name:_____

Site Contact Phone: _____

Site Contact Email: _____

Date:_____

Study: ☐ AA ☐ AAL ☐ COV

Visit (circle one): 1 2 3 4 5 6 7 E

Subject Sex: ☐ M ☐ F

Subject Year of Birth:_____

Tracking #:_____

Blood Collection:

1. Date Drawn (MM/DD/YYYY): _____
2. Time of Drawn (24 hour clock): _____ [HHMM]
3. Last time subject ate (MM/DD/YYYY): _____
4. Last time subject at (24 hour clock): _____ [HHMM]

Blood Processing:

Plasma (3 x 10mL EDTA Lavender Top Tubes)	
Original volume drawn (3x10 mL EDTA tubes): EDTA #1: _____ mL EDTA #2: _____mL EDTA #3: _____mL	
Time spin started (24 hour clock):	_____ [HHMM]
Duration of centrifuge:	_____minutes
Temp of centrifuge:	_____°C
Rate of centrifuge:	_____ xg
Time aliquoted:	_____ [HHMM]
Number of 1.5 mL plasma (purple-cap) aliquots created:	_____
If applicable, volume of residual plasma aliquot (less than 1.5 mL-Blue cap):	_____mL *or* ☐ N/A
If applicable, specimen number of residual plasma aliquot (Last four digits):	_____ *or* ☐ N/A
Time aliquots placed in freezer (24 hour clock):	_____ [HHMM]
Storage temperature of freezer:	_____°C

Whole Blood for DNA (1 x 6mL EDTA Lavender Top Tube)	
Original volume drawn (1x6 mL EDTA tube):	_____mL
Time tube placed in freezer (24 hour clock):	_____ [HHMM]
Storage temperature of freezer:	_____°C

Notes: _____

_____

Version 8.2024