DOD-ADBI Manual of Procedures Update: Version 04.28.2025

Section	Change
	Updated Cover page to include The Unveristy Of Pennsylvania (UPenn) logo.
2.0	Included UPenn information
3.2	Added UPenn Contacts
3.3	Updated to included UPenn hours operation
3.4	Updated to included UPenn Holiday Observations
3.4	NCRAD Winter Break Holiday added
3.5	Removed Abe Ahn and added Jennifer Marcoe as OSHU coordinator contact
4.0	Updated to included UPenn
5.1	Updated centrifuge requirements.
5.2.1	Updated Biofluid Collection Schedule table to list visit numbers.
5.2.2	Noted fasting requirements.
5.2.2	Noted blood collection priorities.
6.0	Updated to included UPenn
7.2	Noted fasting requirements.
7.2	Updated centrifuge requirements and updated process time.
7.2	Updated Plasma & Buffy Coat from EDTA Purple-Top Tube (3x10ml) schematic process time and temperature of centrifuge.
7.2	Removed Set centrifuge to 4°C to pre-chill before use.
7.2	Removed Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.
7.2	Updated centrifuge speed from 2000 x g to 1500 RFC (xg)
7.2	Updated Plasma and Buffy Coat Preparation Schematic
8.3	Added UPenn Frozen Shipping Instructions section
10.0	Updated centrifuge requirements in Appendix B.
10.0	Appendix C: Blood Sample and Shipment Notofication Form for NCRAD has be change. Areas of the form has been removed.

10.0	Added Appendix D: Blood Sample and Shipment Notification Form for UPenn

Department of Defense Alzheimer's Disease Blood Testing Initiative

(DoD-ADBI)

in collaboration with the

National Centralized Repository for Alzheimer's Disease and Related Dementias

and

The University of Pennsylvania

Biospecimen Collection, Processing, and Shipment Manual of Procedures

Version 04.28.2025

Table of Contents

1.0 Abbreviations

AD	Alzheimer's Disease
DoD-ADBI	Department of Defense Alzheimer's Disease Blood Testing Initiative
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
GUID	Globally Unique Identifier
IATA	International Air Transport Association
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
UPenn	University of Pennsylvania
PID	Participant Identifier
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 DoD-ADBI. The purpose of this manual is to provide study staff (PIs, study coordinators, phlebotomists) at the various study sites with instructions for collection and submission of biological samples for study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University and to the University of Pennsylvania located in Philadelphia, PA (UPenn).

Sites will collect and send the following samples to UPenn:

- Plasma

Sites will collect and send the following samples to NCRAD:

- Buffy Coat (DNA Extraction)
- Whole Blood in PAXgene™ tube

This manual includes instructions for the collection of blood, fractionation of blood from collection tubes, aliquoting, labeling, storage prior to shipping, and shipping to NCRAD or UPenn. These procedures are relevant to all study personnel responsible for processing specimens provided to NCRAD and UPenn for the DoD-ADBI protocol.

3.0 NCRAD and UPenn Information

3.1 NCRAD Contacts

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

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

Ronae Williams, MSW, BA Clinical Research Coordinator
Phone: 317-278-9082
Email: rdw2@iu.edu

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

Sample Shipment Mailing Address
NCRAD
Indiana University School of Medicine
351 W. 10th St. TK-342
Indianapolis, IN 46202
Phone: 1-800-526-2839

Study-Specific NCRAD Website page: https://ncrad.org/resource/dod-adbi.html

3.2 UPenn Contacts

Leslie Shaw, PhD, Biomarker Core Director
Phone: 215-662-6575
Email: shawlmj@pennmedicine.upenn.edu

Magdalena Korecka, PhD, Senior Project Coordinator
Phone: 215-662-6266
Email: korecka@pennmedicine.upenn.edu

Yang Wan, MS, Repository Manager
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Sample Shipment Mailing Address

University of Pennsylvania
Department of Pathology and Lab.Med.
Biomarker Core Laboratory
Maloney Building 7th floor
3400 Spruce Street
Philadelphia, PA 19104
phone: 215-662-6266

3.3 NCRAD and UPenn Hours of Operation

Indiana University and University of Pennsylvania 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.

3.4 NCRAD and UPenn 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
December 25	Christmas Day
December 26-31	Winter Break **NCRAD Only**

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://www.ncrad.org/contact/holiday-closures or additional information.**

The University of Pennsylvania is closed for winter vacation from December 25th to January 1st. Shipments of biological materials should be completed **before December 20th.**

- 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.**
- **UPenn does not accept samples during weekends/holidays.**

3.5 DoD-ADBI Contacts

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Jennifer Marcoe, Primary Oregon Health & Science University (OSHU)
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4.0 Globally Unique Identifier (GUID)

The GUID is a participant ID that allows researchers to share data specific to a study participant, without exposing personally identifiable information. A GUID is made up of random alpha-numeric characters and does not include any PHI in the identifier. By using GUIDs in your research data, the system can associate a single research participant's genetic, imaging, and clinical assessment data even if the data was collected at different locations or throughout different studies. No PHI will be sent to NCRAD or UPenn, only the GUID.

To create a GUID follow these steps:

1. Create an account: https://bricsguid.nia.nih.gov/portal/jsp/login.jsp
2. Once you have an account, go to the GUID Tool – Create GUID
3. To open the 'Launch GUID Tool' you will need to have Java installed on your device
4. In order to generate a GUID, the following PHI is required (Appendix A):
    - Complete legal given (first) name of participant at birth
    - If the participant has a middle name
    - Complete legal family (last) name of participant at birth
    - Day of birth
    - Month of birth
    - Year of birth
    - Name of city/municipality in which participant was born
    - Country of birth

5.0 Laboratory Collection

5.1 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
- Wet Ice Bucket
- Wet ice
- Pelleted dry ice

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

- Centrifuge capable of ≥ 1500 x g
- -80°C Freezer

In order to ship specimens, you must provide:

- Pelleted dry ice (approximately 10lbs per shipment)

5.2 Biospecimens Sent to NCRAD or UPenn

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 following tables.

5.2.1 Biofluid Collection Schedule

Timepoint	Plasma	Buffy Coat	RNA
All Visits	**X**	**X**	**X**

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/bank-samples/sample-management/recommended-consent-language. A copy of the consent form for each participant should be kept on file by the site investigator.

5.2.2 Biofluid Collection Chart

Fasting overnight (Minimum 6 hours) is required for plasma collection. Only water is permitted until these blood draws are completed.

Important: Please draw all local clinical lab specimens before research samples for this protocol and then proceed to the listed below draw order.

Draw Order	Collection Tube	Drawn At	Specimen Type	Aliquot Volume	Total Number of Aliquots	Shipping Destination	Shipping Temperature
1	**3 EDTA (Purple-Top) Blood Collection Tubes (10 ml)**	Each visit	Plasma	1.8 ml	1	UPenn	Frozen
			Plasma	0.5 ml	Up to 26	UPenn	Frozen
			Buffy Coat	~1.0 ml	Up to 3	NCRAD	Frozen
2	**1 PAXgene™ Blood Collection Tube (2.5 ml)**	Each visit	Whole Blood	N/A	N/A	NCRAD	Frozen

6.0 Specimen Collection Kits, Shipping Kits, and Supplies

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD and UPenn (for further analysis), the Blood Supplemental Supply Kit, the

Frozen Shipment Kit; 2) including and clinical lab supplies (with the exception of pelleted dry ice and equipment supplies listed in Section 5.1). The provided materials include blood tubes, pipettes, boxes for plasma, buffy coat, and aliquots, as well as shipping materials to send biospecimens to NCRAD or UPenn. Kit number labels, PID labels, and collection tube and aliquot labels will all be provided by NCRAD. Details regarding the blood kits are found in this Manual of Procedures. Collection tubes 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.

6.1 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. Please keep kit contents in original bag provided by NCRAD so supplies are not mixed together.*

Blood Kit

Quantity	Blood Kit Components
1	Large Plastic Bag
1	4x5 Plastic Bag
3	EDTA (purple-top) blood collection tube (10 ml)
1	PAX Gene tube, 2.5ml
1	50 ml conical polypropylene tube (blue cap)
26	Cryovial (2.0 ml) with purple cap
3	Cryovial (2.0 ml) with clear cap
1	Cryovial (2.0 ml) with yellow cap
33	Preprinted Collection Tube and Aliquot Labels
3	Preprinted Kit Number Label
5	Label for handwritten Site and PID
2	Disposable graduated transfer pipettes (3 ml)

Blood Supplemental Supply Kit

Quantity	Blood Supplemental Supply Kit Components
2	Large Plastic Bag
4	4x5 Plastic Bag
6	EDTA (purple-top) blood collection tube (10 ml)
2	PAX Gene, 2.5ml
5	50 ml conical polypropylene tube (blue cap)

Quantity	Blood Supplemental Supply Kit Components
52	Cryovial (2.0 ml) with purple cap
6	Cryovial (2.0 ml) with clear cap
1	Cryovial (2.0 ml) with yellow cap
2	Disposable graduated transfer pipettes (3 ml)
5	Label for handwritten Site and PID
2	Cryovial box (holds up to 25 cryovials)
2	Blue UPS Dry Ice Label
2	UN3373 Sticker
2	Fragile Label
2	Shipping Pouches
2	Small frozen shipper/ Sm brain box
2	Cryobox, 81 cell
2	Resealable tube pouches, on roll
2	Biohazard bag w/ absorbent sheet

NCRAD Frozen Shipping Supply Kit (Small Shippers)

Quantity	Frozen Shipping Kit Components
1	Large Plastic Bag
2	Plastic Biohazard bag with absorbent sheet (small)
6	Resealable tube pouches, on roll
2	Cardboard cryobox, 25 slot
1	UPS Airbill Sleeve
1	Shipping box/Styrofoam container (small)
1	Warning label packet (UN3373 label, pelleted dry ice shipping label and Fragile Label)

UPENN Frozen Shipping Supply Kit (Small Shippers)

Quantity	Frozen Shipping Kit Components
1	Large Plastic Bag
2	Cryobox, 81 cell
2	Large Biohazard shipping bag w/ absorbent sheets
1	Small frozen shipper/Sm brain box
1	Warning label packet (UN3373 label, pelleted dry ice shipping label and Fragile Label)

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module
By Request	Cryovial box (holds up to 25 cryovials)
By Request	Cryobox, 81 cell
By Request	Cryovial (2.0 ml) with purple cap
By Request	Cryovial (2.0 ml) with clear cap
By Request	Cryovial (2.0 ml) with yellow cap
By Request	50 ml conical polypropylene tube (blue cap)
By Request	UPS Airbill Sleeve
By Request	Shipping container for pelleted 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	Plastic biohazard bag with absorbent sheet (Large)
By Request	Disposable graduated transfer pipette (3 ml)
By Request	EDTA (Purple-Top) Blood Collection Tube (10 ml)
By Request	Warning label packet (UN3373 label, pelleted dry ice shipping label and Fragile Label)
By Request	UN3373 label
By Request	Fragile Label
By Request	Biohazard label
By Request	Pelleted dry ice shipping label
By Request	UPS Airbill Sleeve
By Request	Site and PID Labels

6.2 Kit Supply to Study Sites

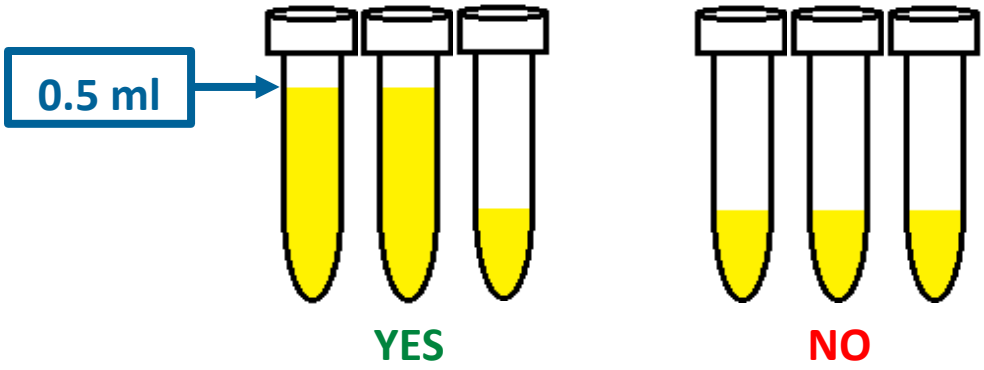
Each site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits. Please go to: http://kits.iu.edu/DoD-ADBI 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.

6.3 Filling Plasma Cryovials

In order to ensure that UPenn 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 biological material as the residual volume and ship to UPenn. Ship *all* plasma to UPenn. Fill as many aliquot tubes as possible. For example, if 1.2 ml of a plasma sample is obtained, fill 2 cryovials with 0.5 ml, and one additional cryovial with the remaining 0.2 ml.



Please note: If there are any unused cryovials, please do not send the empty cryovials to UPenn or 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 following chart summarizes the association between cap color and type of aliquot.

Cap Color	Sample Type
Yellow	Plasma for C2N
Purple	Plasma for UPenn
Clear	Buffy Coat for NCRAD

7.0 Blood Collection and Processing Procedures

7.1 Labeling Samples

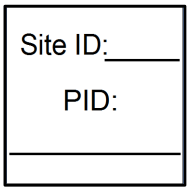
In order to ensure the highest quality samples are collected, it is essential to follow the specific collection and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and prepared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

7.1.1 Label Type Summary

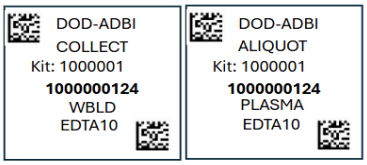
1. Kit Number Label
2. Site and PID Label

3. Collection Tube and Aliquot Label

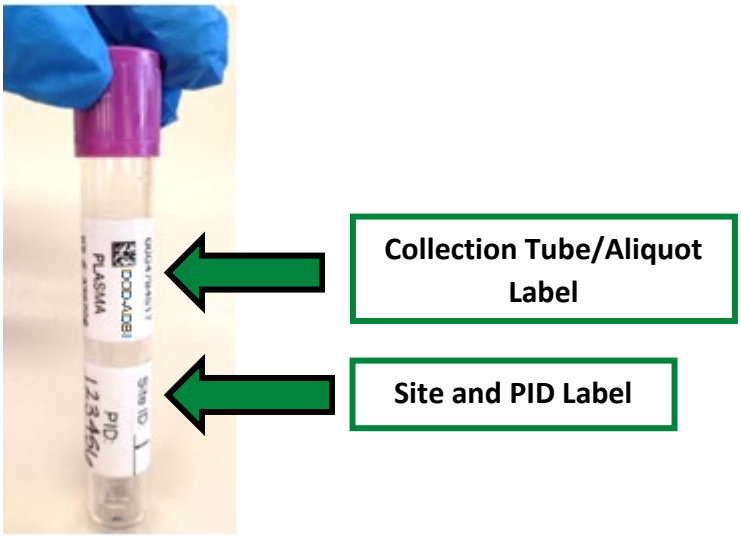
Kit Number Labels tie together all specimens collected from one participant at one visit. They should be placed on each cryobox, and in the designated location on the Blood Sample and Shipment Notification Forms.



Site and PID Labels are used to document the individual's unique Site and PID. Place one label on each blood collection tube.



Place one **Collection Tube and Aliquot Label** on each blood collection tube and cryovial.



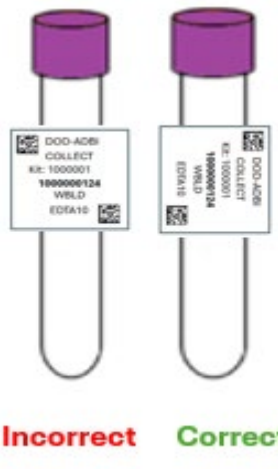
Labeled EDTA (Purple-Top) Blood Collection Tube

Each collection tube will contain two labels: the collection tube label and the Site and PID 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 PID label near the bottom of the tube.

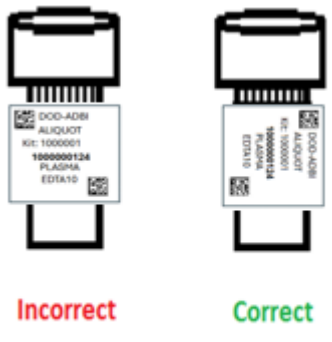
In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place Collection Tube and Aliquot Labels on **ALL** collection tubes and 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 Site and PID 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. The following pictures show the correct orientation of the labels on the collection tubes and cryovials.



Collection Tube Label Diagram



Aliquot Label Diagram

7.2 Whole Blood Collection with 10 ml EDTA (Purple-Top) Tube for Plasma and Buffy Coat

Whole Blood Collection for Isolation of Plasma and Buffy Coat: three EDTA (Lavender-Top) Blood Collection Tubes (10 ml) (for processing of plasma aliquots and buffy coat aliquots).

Fasting overnight (Minimum 6 hours) is required for plasma collection. Only water is permitted until these blood draws are completed.

1. Store empty EDTA tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use. Check expiration dates on all collection tubes before visit.

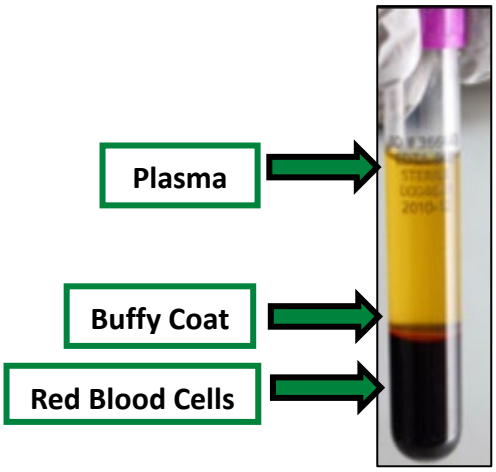
2. Place completed Site and PID Label and preprinted **PLASMA** Collection Tube Label on the purple-top EDTA tubes. Place preprinted **PLASMA** Aliquot Labels on the 2 ml cryovials with purple AND **yellow** caps and 2 ml cryovial with blue cap (if necessary, for residual). Place preprinted **BUFFY COAT** Aliquot Label on the 2 ml cryovials with clear caps.
3. Using a blood collection set and a holder, collect blood into the **EDTA (Purple-Top) Blood Collection Tube (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 last collection tube.
    d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

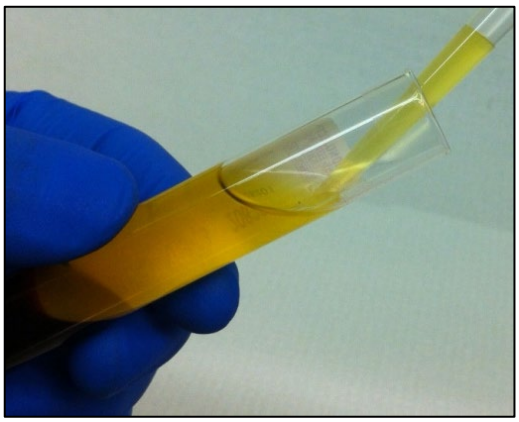
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
    a. If complications arise during the 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 tube 8-10 times.

6. Centrifuge balanced tubes for 15 minutes at 1500 RCF (x g) at Room Temperature. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix B to calculate equivalent RPM for spin at 1500 RCF (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 **60 minutes** from the time of collection.
   d. Record time aliquoted on the Biological Sample Shipment and Notification Form.

7. 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.

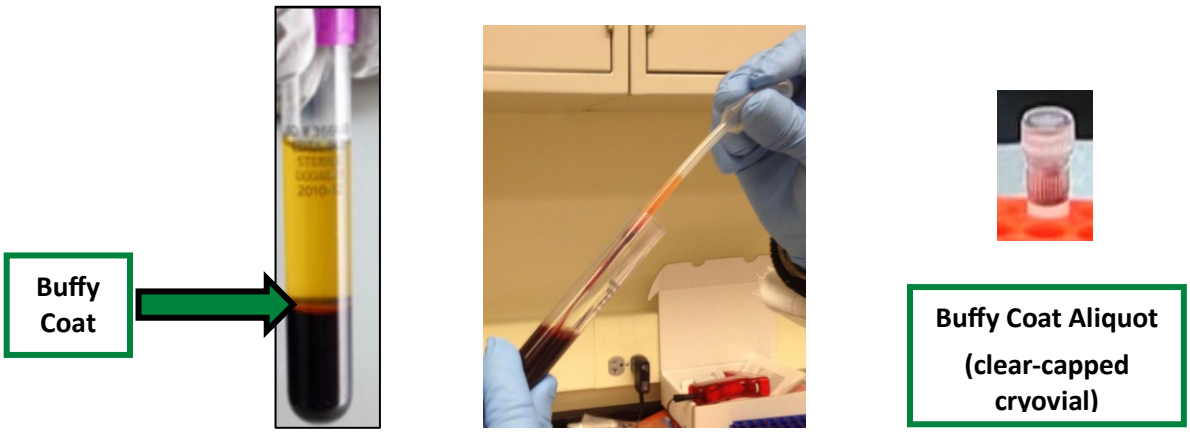




8. Each EDTA tube should yield, on average, 4-5 ml of plasma. Transfer plasma from all EDTA tubes into the 50 ml conical tube and gently invert 3 times. **When pipetting plasma from the EDTA tube into the 50 ml conical tube, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched. First aliquot 1.8 ml of plasma into the one yellow-capped cryovial.** Then aliquot the remaining plasma in 0.5 ml increments in the remaining purple-capped cryovials. Be sure to only place **plasma** in cryovials labeled with **PLASMA** labels**.**

9. Place the labeled plasma cryovials in the 81 cell cryobox and place on pelleted dry ice. **Transfer to -80°C Freezer when possible**. Store all samples at **-80°C until shipped** to UPenn on pelleted dry ice. Record time aliquots frozen and storage temperature of freezer on UPenn's Biological Sample Shipment and Notification Form (Appendix D)

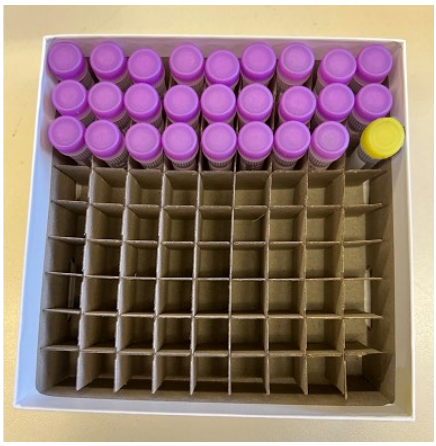
10. After plasma has been removed from the EDTA (Purple-Top) Blood Collection Tubes (10 ml), aliquot the buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) from one EDTA tube into a labeled, clear-capped cryovial using a micropipette. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to only place the buffy coat from one EDTA tube into each cryovial. Repeat this step for the second and third EDTA tubes, placing these buffy coats into the second and third clear-capped cryovials.



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

12. Record the specimen number and volumes of the EDTA tubes and corresponding buffy coat samples on the Biological Sample Shipment and Notification Form.

13. Place the labeled cryovials in the 25 cell cryobox and place on pelleted dry ice. **Transfer to -80°C Freezer when possible.** Store all samples at -80°C until shipped to NCRAD on pelleted dry ice. Record time aliquots frozen and storage temperature of freezer on NCRAD's Biological Sample and Shipment Notification Form (Appendix C) .



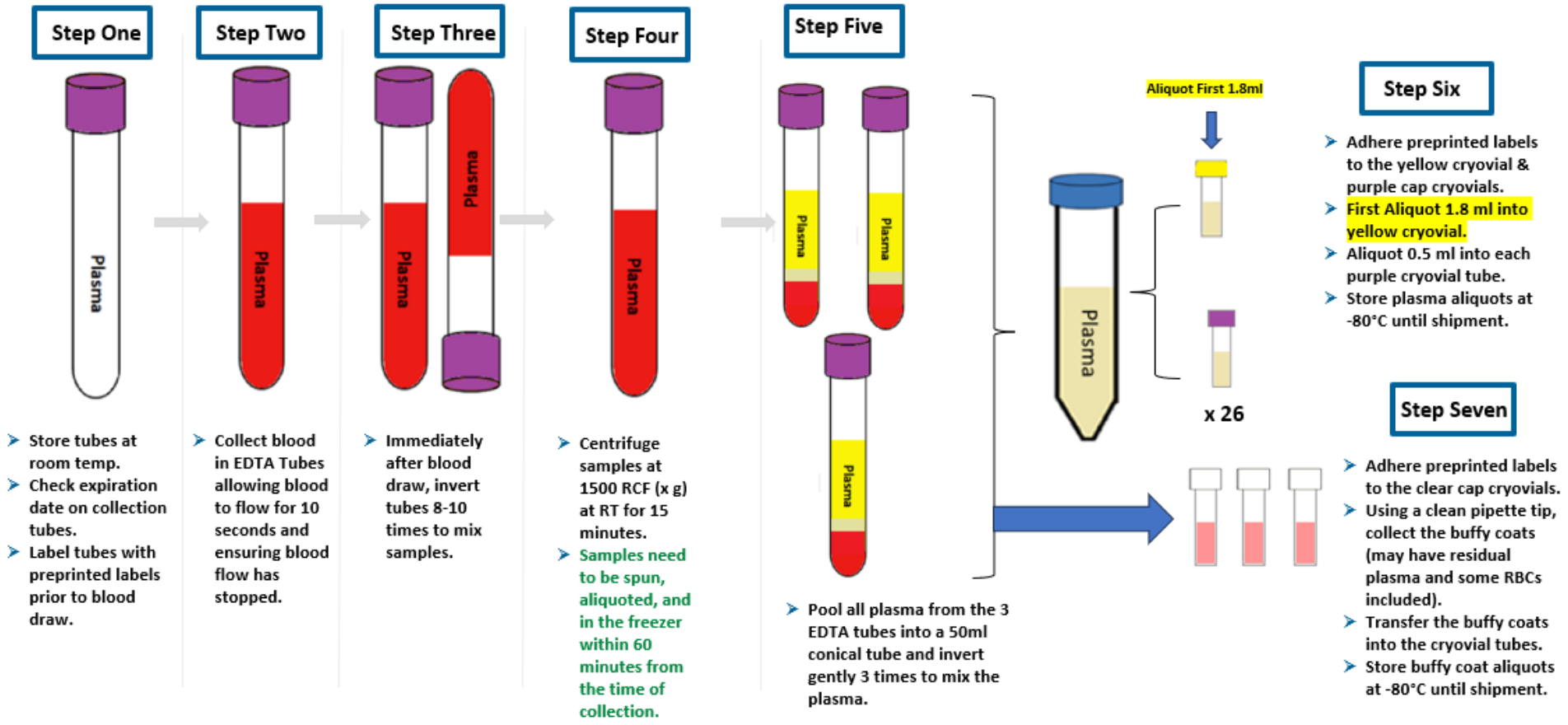
Plasma Aliquots (up to 27) per each study participant for shipment to UPenn

Up to 2 Study Participants per cryobox

Buffy Coats (3) per each study participant for shipment to NCRAD

Up to 3 Study Participants per cryobox

Plasma and Buffy Coat Preparation (10ml Lavender-Top Tube x 3)



- Store tubes at room temp.
- Check expiration date on collection tubes.
- Label tubes with preprinted labels prior to blood draw.

- Collect blood in 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 samples at 1500 RCF (x g) at RT for 15 minutes.
- Samples need to be spun, aliquoted, and in the freezer within 60 minutes from the time of collection.

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

- Adhere preprinted labels to the yellow cryovial & purple cap cryovials.
- First Aliquot 1.8 ml into yellow cryovial.
- Aliquot 0.5 ml into each purple cryovial tube.
- Store plasma aliquots at -80°C until shipment.

- Adhere preprinted labels to the clear cap cryovials.
- Using a clean pipette tip, collect the buffy coats (may have residual plasma and some RBCs included).
- Transfer the buffy coats into the cryovial tubes.
- Store buffy coat aliquots at -80°C until shipment.

7.3 PAXgene™ Blood Collection Tube (2.5 ml) for RNA

Whole Blood Collection for Isolation of RNA: one PAXgene™ Blood Collection Tubes for RNA.

1. Store PAXgene™ Blood Collection Tubes at room temperature 64°F - 77°F (18°C to 25°C) before use. Check expiration dates on all collection tubes before visit.

2. Place completed Site and PID label and "**RNA**" collection tube label on the PAXgene™ Blood Collection Tubes (2.5 ml) prior to blood draw; no processing is required for these tubes**; the tubes are to be shipped to NCRAD frozen, without processing at the collection site.**

3. Using a blood collection set and a holder, collect blood into the **one PAXgene™ Blood Collection Tube** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

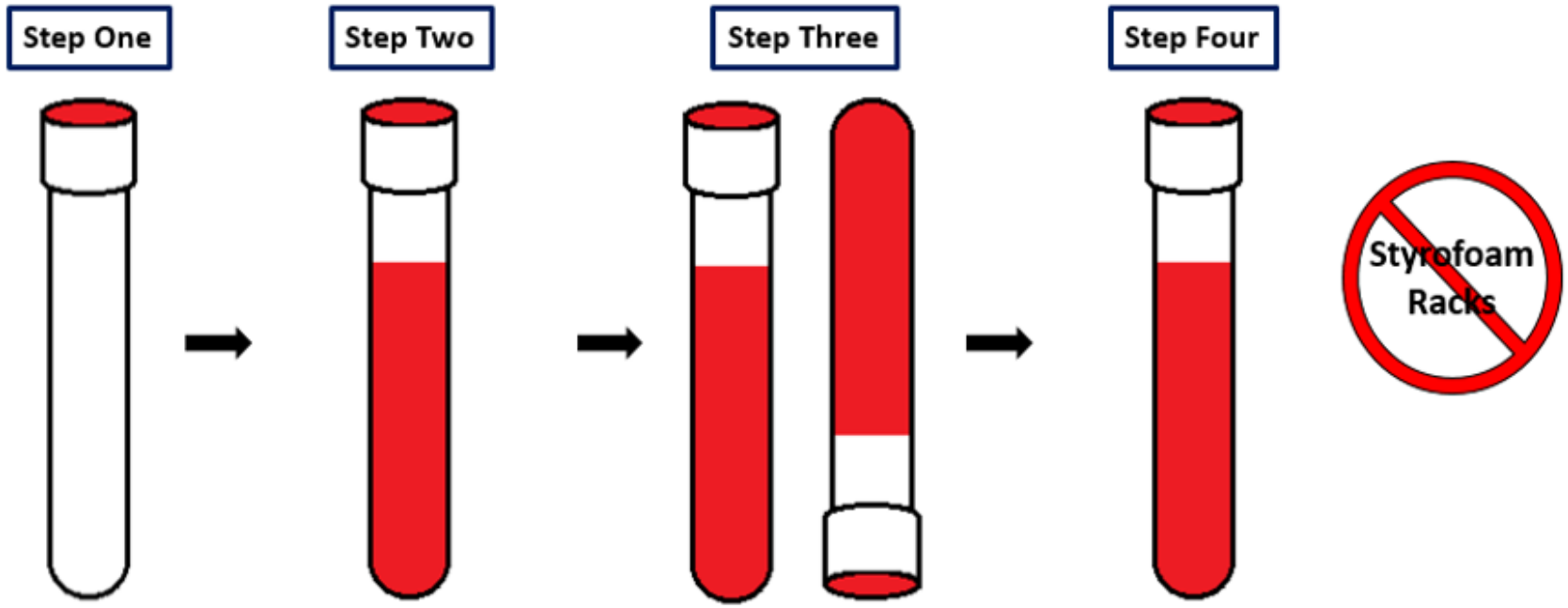
   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into last collection tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

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 PAXgene™ Blood RNA Tube with its vacuum is designed to draw 2.5ml of blood into the tube. Record total amount of blood drawn into PAXgene™ blood tube(s) within the Biological Sample and Shipment Notification Form.

5. Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene™ Blood RNA Tubes 8 – 10 times.

6. Place the PAXgene™ tubes upright in a **WIRE** or **PLASTIC** rack. Transfer to **-80°C Freezer within two hours of the draw**. Record vial location and freezer on batch record. Store all samples at **-80°C until shipped** to NCRAD on pelleted dry ice. **Do NOT use a Styrofoam rack. This** will cause the PAXgene™ tubes to crack. Complete remainder of the Biological Sample and Shipment Notification Form (Appendix B).

RNA Preparation (2.5ml PAXgene™ Tube x 1)



- Store tubes at room temp.
- Check expiration date on collection tubes.
- Label tubes with preprinted labels prior to blood draw.

- Collect blood in PAXgene™ tube allowing blood to flow for 10 seconds, and ensuring blood flow has stopped.

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

- Store tubes in wire rack at -80°C until shipment to NCRAD.
- Do not store tube in Styrofoam racks.

8.0 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 number of samples received are tracked by NCRAD and UPenn 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.

8.1 Frozen Packaging Instructions

FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY!

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.

Small Frozen Shipper:

**10 lbs of dry ice pellets

AND

- Up to 2 x 25-cell cryoboxes

AND

- Up to 6 x PAXgene™ tubes

Shipment to NCRAD

Small Frozen Shipper:

**10 lbs of dry ice pellets

AND

- Up to 2 x 81-cell cryoboxes

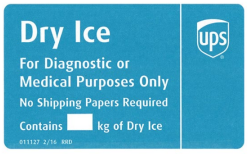
Shipment to UPenn

Specimens being shipped to NCRAD or UPenn 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 pelleted dry ice contained



8.1.1 NCRAD Packaging Instructions – Frozen Shipments

1. 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.

2. Notify NCRAD or UPenn of shipment by emailing NCRAD coordinators at alzstudy@iu.edu or the UPenn Repository Manager at Yang.Wan@pennmedicine.upenn.edu with "DoD ADBI" in the subject line in include the UPS tracking number.

3. Attach the completed Sample Form (Appendix C or Appendix D) to the email notification.
    a. If email is unavailable please call NCRAD or UPenn and do not ship until you've contacted and notified NCRAD or UPenn coordinators about the shipment in advance.
    b. UPenn requires an electronic version of the shipping manifest to be also sent by email with **DoD ADBI** in the subject line.

4. Place the cryovial boxes containing frozen samples into a biohazard bag.

5. As the cryovial box is placed in the plastic biohazard bag, do NOT remove the absorbent material found in the bag. Seal according to the instructions on the bag.

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

7. Place the biohazard bags into the provided Styrofoam-lined shipping container on top of the pelleted dry ice. Please ensure that cryovial boxes are placed so the cryovials are upright in the shipping container. **A maximum of 2 cryoboxes and 6 PAXgene™ tubes may be sent in each shipper.**

8. 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.

9. 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.

10. Complete the UPS Dry Ice Label with the following information:

a. Net weight of pelleted 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.

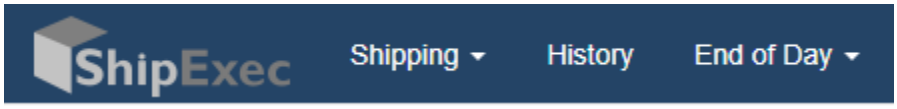
11. Apply all provided warning labels and UPS return airbill to the outside of package, taking care not to overlap labels. **Complete the required fields on the UPS Dry Ice label or UPS may reject or return your package.**

12. Use UPS tracking to ensure the delivery occurs as scheduled and is received by NCRAD or UPenn.

8.2 Frozen Shipping Instructions for NCRAD Shipments

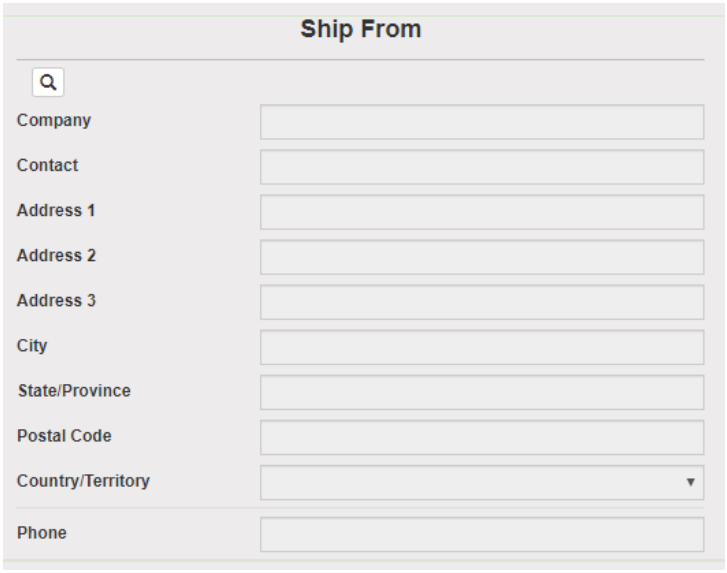
1. Log into the ShipExec Thin Client at kits.iu.edu/UPS.
    a. If a new user or contact needs access, please reach out to your study contact for access.

2. Click “Shipping” at the top of the page and select “Shipping and Rating”.



3. Select your study from the “Study Group” drop down on the right side of the main screen. Choosing your study will automatically filter the address book to only addresses within this study.

4. 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.

5. 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.
6. 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.

7. 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.

8. 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.

9. Fold airbill, and place inside plastic UPS sleeve.

10. Peel the back off of the UPS sleeve and stick the sleeve to the package top. Ensure that sleeve does not cover any warning labels (e.g. dry ice label) or overlap taped seams.

8.3 Frozen Shipping Instructions for UPenn Shipments

1. NCRAD will include preprinted UPS airbills for shipments to UPenn in the UPenn Frozen Shipping Kits. Once the frozen shipment package is prepared, peel of the backing of the airbill and adheard the label onto the shipping box. Please ensure that the UPS airbill sticker does not cover any of the warning labels (e.g. dry ice label or UN3373) or overlap taped seams.

2. It is important to ensure that the dry ice weight on the preprinted airbill matches the dry ice weight on the on the blue UPS dry ice label and what is included in the package.

9.0 Data Queries and Reconciliation

Sample and Shipment Notification forms must be completed on the day that samples are collected because they include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

NCRAD and UPenn will collaborate with the data team at DOD-ADBI to reconcile information captured in the DOD-ADBI database compared to samples received and logged at NCRAD or UPenn. Additional discrepancies may be sent directly to the center staff to reconcile.

Data queries or discrepancies with samples shipped and received at NCRAD or UPenn 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 or UPenn compared to information entered into the DOD-ADBI database.

10.0 Appendices

Appendix A: GUID Demographics Form

Appendix B: Rate of Centrifuge Worksheet

Appendix C: NCRAD Blood Sample and Shipment Notification Form

Appendix D: UPenn Blood Sample and Shipment Notification Form

Appendix A: GUID Demographics Form

Please be certain to collect the following demographic information to generate a Global Unique Identifier. **Do NOT** return this information to NCRAD or UPenn. Only send the GUID to NCRAD and UPenn.

1. Compete legal given (first) name of participant at birth: _____
2. Complete additional (middle) name or names at birth: _____
3. Complete legal family (last) name of participant at birth: _____
4. Suffix: _____
5. Date of Birth: _____
6. Name of city/municipality in which participant was born: _____
7. Country of birth: _____

Appendix B: Rate of Centrifuge Worksheet

Please complete and return this form by 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).

Within 60 minutes of blood collection, centrifuge balanced tubes for 15 minutes at 1500 RCF (x g)

Your centrifuge's RCF can be determined using the formula below:

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

- Fixed angle rotor: Distance from center of the turning axis to the bottom of the centrifuge
- Swing Bucket Rotor: Distance from center of the turning axis to the middle of the bucket

Refrigeration prior to or during centrifugation is not recommended. Sites are responsible for ensuring centrifuge has correct settings.

Comments:

Please send this form to NCRAD Study Coordinator at alzstudy@iu.edu

Appendix C: NCRAD Blood Sample and Shipment Notification Form

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

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

From:_____ **UPS tracking #:** **1Z976R8W84**

Phone:_____ **Email:**_____

Study: DoD-ADBI **Sex:** ☐M ☐F **Year of Birth:** _____

Site ID: _____ **PID**: _____

GUID: _____ **Visit:**_____

KIT BARCODE

Blood Collection:

Date of Draw: _____ [MMDDYY]	Time of Draw: _____ [HHMM]
Date participant last ate: _____ [MMDDYY]	Time participant last ate: _____ [HHMM]

RNA (PAXgene™ Tubes)

Original volume drawn (2.5 ml PAXgene™ tube):	_____ ml
PAXgene™ tubes Time frozen:	_____ [HHMM]

Blood Processing: **Buffy Coat (EDTA Tube)**

EDTA #1 specimen number **(Last four digits):**	_____	Original blood volume of EDTA #1:	_____ mL
EDTA #2 specimen number **(Last four digits):**	_____ ☐N/A	Original blood volume of EDTA #2:	_____ mL ☐N/A
EDTA #3 specimen number **(Last four digits):**	_____ ☐N/A	Original blood volume of EDTA #3:	_____ mL ☐N/A
Buffy coat #1 specimen number **(Last four digits):**	_____	Buffy coat #1 volume:	_____ mL
Buffy coat #2 specimen number **(Last four digits):**	_____ ☐N/A	Buffy coat #2 volume:	_____ mL ☐N/A
Buffy coat #3 specimen number **(Last four digits):**	_____ ☐N/A	Buffy coat #3 volume:	_____ mL ☐N/A
Time aliquots frozen:	_____ [HHMM]	Storage temperature of freezer:	_____ °C

Notes:_____

Appendix D: UPenn Blood Sample and Shipment Notification Form

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

To: Yang Wan (UPenn) Email: Yang.Wan@pennmedicine.upenn.edu Phone: 215-662-3413

From:_____ **UPS tracking #:**_____

Phone:_____ **Email:**_____

Study: DoD-ADBI **Sex:** ☐M ☐F **Year of Birth:** _____

Site ID: _____ **PID**: _____

GUID: _____ **Visit:** _____

KIT BARCODE

Blood Collection:

Date of Draw: _____ [MMDDYY]	Time of Draw: _____ [HHMM]
Date participant last ate: _____ [MMDDYY]	Time participant last ate: _____ [HHMM]

Blood Processing: **PLASMA (3 EDTA Tubes)**

EDTA #1 specimen number **(Last four digits):**	_____	Original blood volume of EDTA #1:	_____ mL
EDTA #2 specimen number **(Last four digits):**	_____ ☐N/A	Original blood volume of EDTA #2:	_____ mL ☐N/A
EDTA #3 specimen number **(Last four digits):**	_____ ☐N/A	Original blood volume of EDTA #3:	_____ mL ☐N/A
Total volume of collected plasma:	_____ mL		
Time spin started:	_____ [HHMM]	Duration of centrifuge:	_____ mins
Temp of centrifuge:	_____ °C	Rate of centrifuge:	_____ RCF (x g)
Time aliquoted:	_____ [HHMM]		
Time aliquots frozen:	_____ [HHMM]	Storage temperature of freezer:	_____ °C

Notes:_____