

HALS Manual of Procedures Update: Version 02.2024

Section	Change
Document Footer	The version date was updated for this amendment.
Throughout	Updated colors, logos
Throughout	Added accessibility text to pictures
Throughout	Changed “subject” to “participant”
3.1	Added phone numbers and new website
3.3	Added Juneteenth to list of NCRAD holiday observations
5.2	Changed “tiger top SST” to “red top serum”; changed to some aliquots kept at Brown
5.4	Updated number of cryovials sent to NCRAD
6.1	Changed from 8 to 7 purple cryovials, removed cryobox from blood kit, added cryoboxes to frozen shipping kit. Removed longitudinal and biofluid visits.
6.2	Changed “two weeks” to “three weeks”
7.0	Updated to “Release tourniquet as soon as blood starts to flow into last collection tube”
7.3	Added: Check expiration dates on all collection tubes before visit. Changed number of EDTA tubes and aliquots sent to NCRAD. Updated processing schematic. Added step 1 to frozen shipping instructions: “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.” Changed 15 minutes to 10 minutes. Removed longitudinal and biofluid visits.
8.0	Updated number of plasma and buffy coat aliquots sent to NCRAD
8.1	Changed “dry ice” to “pelleted dry ice.” Updated frozen shipping instructions. Added step 1 to frozen shipping instructions.

Healthy Aging Across the Lifespan (HALS) at Brown University
in collaboration with the
**National Centralized Repository for
Alzheimer’s Disease and Related
Dementias**



**Biospecimen Collection, Processing, and Shipment Manual of
Procedures**

Version 02.2024

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1.0 Abbreviations

CITI	Collaborative Institutional Training Initiative
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
HALS	Healthy Aging Across the Lifespan
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
NCRAD	National Centralized Repository for Alzheimer’s Disease and Related Dementias
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

2.0 Purpose

The purpose of this manual is to provide the Healthy Aging Across the Lifespan (HALS) staff (PIs, study coordinators, and the sample collection and processing teams) with instructions for collection and submission of biological samples for HALS study visits. It includes instructions for biospecimen submission to the National Centralized Repository for Alzheimer’s disease and Related Dementias (NCRAD) located at Indiana University. The following samples may be collected at each study visit:

- Plasma
- Buffy Coat (for 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 blood specimens to be submitted to NCRAD for the HALS 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

Corinne Kim, MA, CCRC, Study Coordinator

Phone: 317-274-7417

Email: kimcor@iu.edu

General NCRAD Contact Information

Phone: 1-800-526-2839 or 317-278-8413

Email: alzstudy@iu.edu

Website: www.ncrad.org

HALS Study Specific Webpage: <https://ncrad.org/coordinate-studies/hals>

Sample Shipment Mailing Address

HALS at NCRAD

Indiana University School of Medicine

351 W. 10th St TK-217

Indianapolis, IN 46202

Phone: 1-800-526-2839

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 frozen 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
3 rd Monday in January	Martin Luther King, Jr Day
4 th Monday in May	Memorial Day
June 19	Juneteenth (observed)
July 4	Independence Day (observed)
1 st Monday in September	Labor Day
4 th Thursday in November	Thanksgiving
4 th Friday in November	Friday after Thanksgiving
December 25	Christmas 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 at all 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 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, 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 C](#)):
 - 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 HALS 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

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 $\geq 2000 \times g$ with refrigeration to 4°C
- -80°C Freezer

In order to batch-ship frozen specimens, you must provide:

- Pelleted dry ice (approximately 30-45 lbs per shipment)

5.2 Biospecimens Sent to NCRAD

Frozen samples are to be submitted according to the shipping methods outlined in [Section 7.1](#). Guidelines for the processing, storage location, and timing of sample collection are listed by site in the tables below.

Biospecimens collected include: whole blood (plasma and buffy coat as well as serum isolated on-site).

<u>Specimen Type</u>	<u>V01</u>	<u>Shipped to NCRAD</u>	<u>Stored at Brown</u>
Serum	X		X
Plasma	X	X	X
DNA (Buffy Coat)	X	X	X

Whole blood will be collected into two different collection tubes (lavender-top EDTA plasma and red top serum)

The lavender-top EDTA tubes are processed locally into plasma and buffy coat fractions, aliquoted, frozen at the study site, and then some are stored at Brown and some are shipped to NCRAD.

The red top serum tube is processed locally into serum fractions, aliquoted, frozen at the study site and is not returned to NCRAD. Aliquots are stored at Brown.

5.3 Informed Consent

Consent forms must specify that any biological samples and de-identified clinical data may be shared with academic and/or industry collaborators through NCRAD. A copy of the consent form for each participant should be kept on file by the site investigator.

5.4 Biospecimen Collection Charts

5.4.1 Blood Collection

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the **Biological Sample and Shipment Notification Form** (see [Appendix A](#)). Submit a copy to NCRAD with a reason provided for the omission.

Sample Type	Tube Type	Study Visits Collecting Biospecimens	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Typical # of tubes sent to NCRAD	Shipping Temperature to NCRAD
Whole blood for isolation of serum	Serum (Red Top) Blood Collection Tube (10 ml)	V01	1	N/A	0	N/A
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	V01	2	N/A	0	N/A
	PLASMA: 2 ml cryovials with lavender caps (residual volume placed in 2 ml cryovial with blue cap)	V01	3 (2 Lavender Cap, 1 Blue Cap Cryovial)	1.5 ml plasma aliquots per 2.0 ml cryovial	2-3	Frozen
	PLASMA: 2 ml cryovials with lavender caps	V01	4	1.5 ml plasma aliquots per 2.0 ml cryovial	0	N/A
	BUFFY COAT: 2 ml cryovial with a clear cap	V01	2	1 ml buffy coat aliquot per 2.0 ml cryovial	1	Frozen

* Please refer to the table in [Section 4.2](#) for another view of the specimen collection schedule

6.0 Specimen Collection Kits, Shipping Kits and Supplies

There are 3 HALS research kits:

- Blood Kit
- Frozen Shipping Supply Kit
- Supplemental Supply Kit

Research specimen collection kits as well as clinical lab supplies (except pelleted dry ice and equipment supplies listed above in [Section 4.1](#)) will be provided by NCRAD. These materials include blood tubes, boxes for plasma/buffy coat aliquots storage and shipment, as well as partially completed shipping labels to send materials to NCRAD. Barcoded kit labels, collection tube labels, and aliquot tube labels will all be provided by NCRAD. Collection tube labels and aliquot tube labels will be pre-printed 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 6.1](#).

6.1 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.

HALS Blood Kit

Quantity	HALS Blood Kit Components
2	EDTA (Lavender-Top) Blood Collection Tube (10ml)
1	Serum (Red Top) Blood Collection Tube (10ml)
1	15ml conical tube
7	Cryovial tube (2 ml) with lavender cap
2	Cryovial tube (2 ml) with clear cap
1	Cryovial tube (2 ml) with blue cap
4	Disposable graduated transfer pipette
15	Pre-printed labels for blood collection and aliquot tubes
3*	Pre-printed labels with kit number

*1 label for frozen shipment form, 1 label for cryobox, 1 label for small plastic Biohazard Bag

Frozen Shipping Supply Kit

Quantity	Frozen Shipping Kit Components
8	Small plastic Biohazard bag with absorbent sheets
1	Shipping box/Styrofoam container
8	25-slot cryoboxes
1	Warning label packet with dry ice sticker

HALS Supplemental Supply Kit

Quantity	HALS Supplemental Kit Components
5	Cryovial tube box (holds up to 25 cryovials)
5	Small plastic Biohazard bag with absorbent sheet
5	Cryovial tube (2 ml) with blue cap
30	Cryovial tube (2 ml) with lavender cap
16	Cryovial tube (2 ml) with clear cap
5	15ml conical tube
10	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
5	Serum (Red-Top) Blood Collection Tube (10ml)
5	Disposable graduated transfer pipette (3ml)
5	Warning label packet

We realize there may be instances where additional supplies are needed; therefore, one supplemental kit will be provided with the initial kit shipment. Replacement supplemental kits can be requested on the kit web site. In addition, individual supplies can be requested. The following table lists all individual supplies that can be requested and the quantities at which they are available.

Individual Supplies*

Quantities	Item
5 or 10	25 Cell Cryobox
10, 25	Cryovial tube (2 ml) with blue cap
25, 50	Cryovial tube (2 ml) with lavender cap
25, 50	Cryovial tube (2 ml) with clear cap
1, 2, 3	Shipping container for dry ice shipment (shipping and Styrofoam box)
5, 10	Small biohazard bag with absorbent sheet
5, 10, 15	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
5, 10, 15	Serum (Red-Top) Blood Collection Tube (10ml)
30, 60	Disposable graduated transfer pipette
5, 10	Warning label packet

*Available upon request on the kit web-site

6.2 Kit Supply at Study Sites

Each individual 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 so you are prepared for study visits; it can take 3 weeks or more (dependent on volume) for requested kits/supplies to be delivered to you. Please go to: <http://kits.iu.edu/HALS> to request additional kits and follow the prompts to request the desired supplies.

Options include ordering specific number of kits (HALS Blood Kit, Frozen Shipping Supply Kit and/or a Supplemental Supply Kit) or individual supplies.

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

7.0 Blood Collection and Processing Procedures

*****Important Note*****

In order to ensure the highest quality samples are collected, processed, and stored, it is essential to follow the specific collection, processing, 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. Draw blood in the following order:

- 1. Serum (Red Top) Blood Collection Tube (10 ml) for on-site use only**
- 2. EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Buffy Coat and Plasma (2)**

SPECIFIC INSTRUCTIONS FOR COLLECTION AND PROCESSING OF EACH SAMPLE ARE DETAILED ON THE FOLLOWING PAGES.

7.1 Labeling Samples for NCRAD

****Label Type Summary****

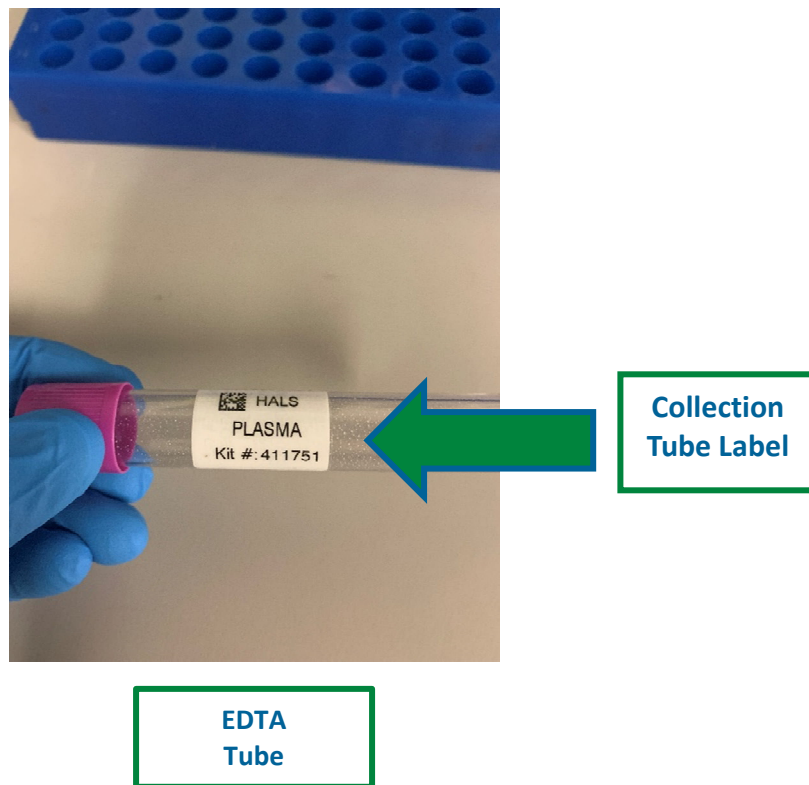
1. Kit Number Label
2. Collection and Aliquot Tube Labels



Kit Number Labels link all specimens collected from a single participant at one visit. They should be placed in the designated location on the Sample and Shipment Notification Form (Blood). The Kit Number Label will also need to be placed on the corresponding patient’s cryovial box, which can be used for storage and is required for sending as a frozen BATCH shipment to NCRAD.

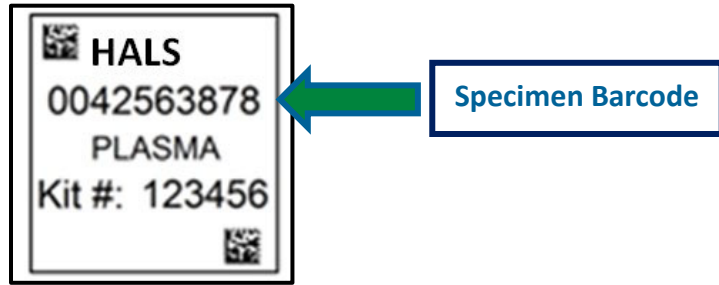


Place the **Collection and Aliquot Tube Labels** on the collection tube and/or the aliquot tube. Each collection tube will contain the **Collection and Aliquot Tube Label** for QC purposes during processing. (Pictured below).

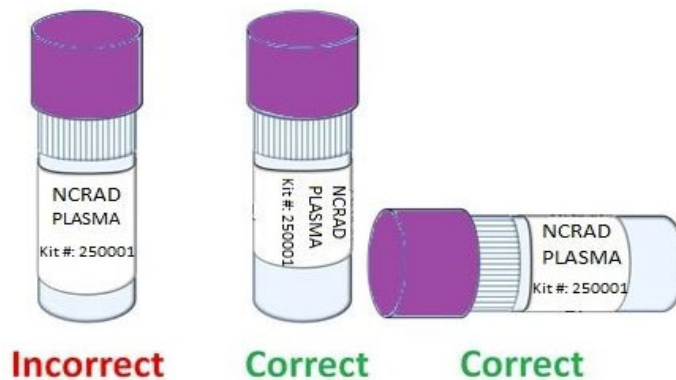


In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place Specimen Labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing, or freezing.
- Place cryovials in cryovial box in numerical order **based on the specimen 10-digit barcode**, located at the top of the label. This ensures that no aliquot is misplaced or lost during the shipment process (see following depiction).



- For best adhesion results place the label on the tubes **BEFORE** sample collection, processing, or freezing.
- The Collection and Aliquot Tube Labels contain a 2D barcode on the left-hand side of the label. Place this barcode toward the tube cap.
- Always place the label **horizontally** on the aliquot tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see following labeling diagram).



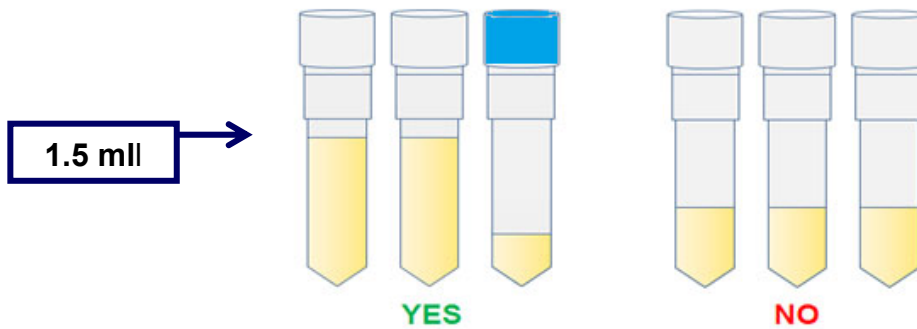
- Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label. Start first near the base of the label closest to the kit number and work your way around, ensuring that the label wraps completely around itself.

7.2 Filling Aliquot Tubes (Plasma)

In order to ensure that NCRAD receives enough 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 into a blue-cap residual tube and ship to NCRAD. Ship *all* material to NCRAD outline in the processing schematic. Fill as many aliquot tubes as possible. For example, if 2.7 ml of a plasma sample is

obtained, fill 1 cryovial tube with 1.5 ml, and one additional cryovial tube with the remaining 1.2 ml. Do not exceed 1.5 ml in any single aliquot tube.



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). 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’s 10-digit barcode 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
Clear	Buffy Coat
Blue	Residual Aliquot

7.3 EDTA (Lavender-Top) Blood Collection Tube (10 ml) 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). Three lavender-top tubes are collected at every study visit obtaining biospecimens.

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. Set centrifuge 4°C to pre-chill before use. Please note that the centrifuge could take 30 minutes to chill completely.
3. Place “**PLASMA**” *collection tube label* on the two EDTA (Lavender-Top) Blood Collection Tubes (10 ml). Place “**PLASMA**” *aliquot labels* on the (~3) 2 ml cryovial tubes with lavender caps. Place pre-printed “**BUFFY COAT**” aliquot label on the two 2 ml cryovials with a clear cap.
4. Using a blood collection set and a holder, collect blood into the **two 10 ml EDTA tubes** using your institution's recommended procedure for standard venipuncture technique.

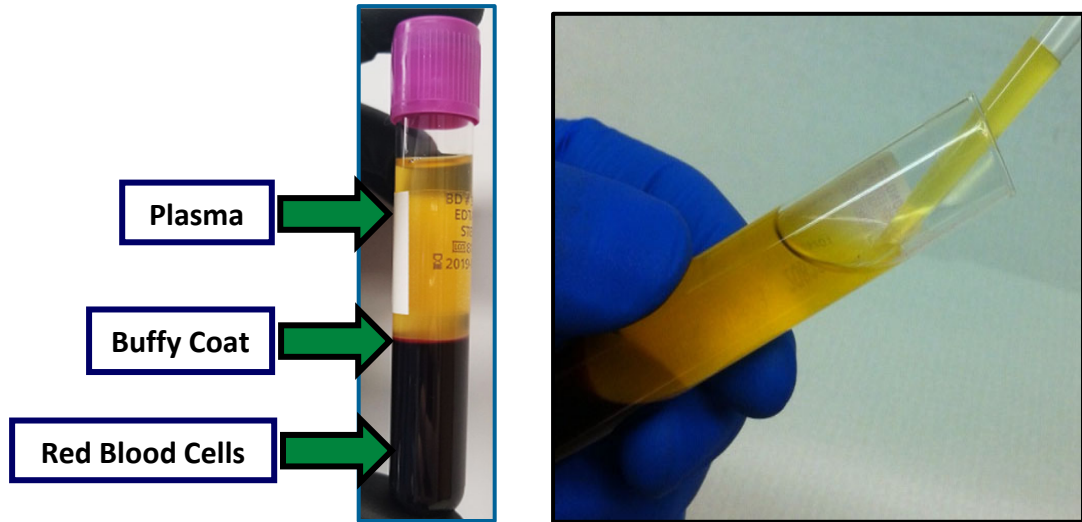
The following techniques shall be used to prevent possible backflow:

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

7. Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.

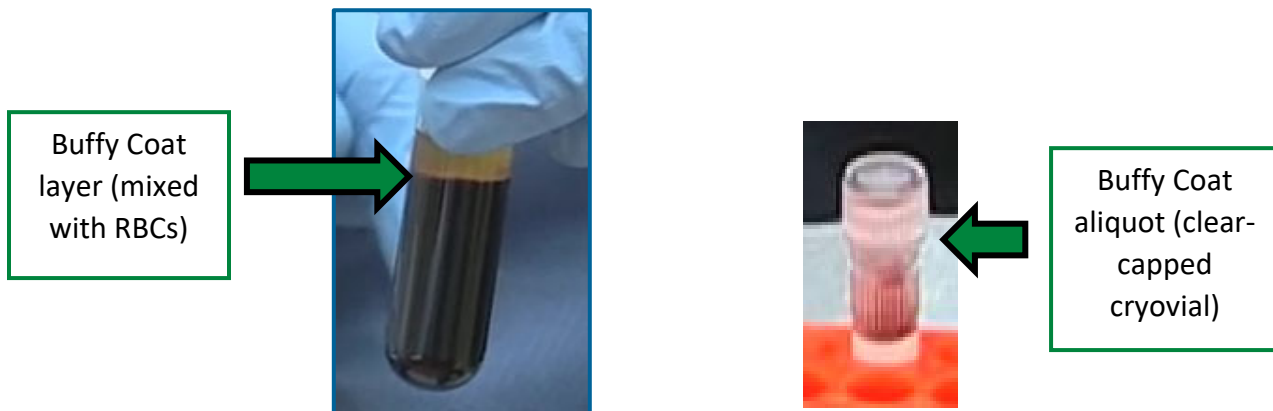


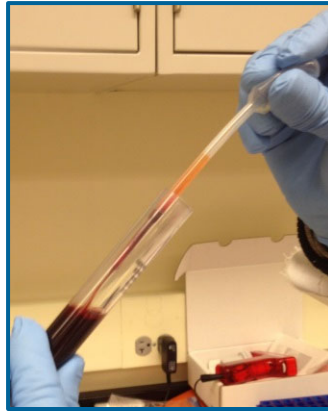
8. Centrifuge balanced tubes for 10 minutes at 2000 RCF (x g) at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation.**
 - a. Equivalent rpm for spin at 2000 x g
 - b. While centrifuging, remember to record all times, temperatures and spin rates on the Blood Sample and Shipment Notification Form.
 - c. Record time aliquoted on the Blood Sample Shipment and Notification Form.
 - d. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
9. 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 blood cells at the bottom of the collection tube.
10. Each EDTA tube should yield, on average, 4-5 ml of plasma. Using a disposable graduated transfer pipette, transfer plasma from both EDTA tubes into a 15 ml conical tube and gently invert 3 times. Aliquot 1.5 ml plasma per cryovial. Be sure to only place **plasma** in cryovials with purple caps and labeled with **PLASMA** labels. Place residual plasma (<1.5 ml) in the blue-capped cryovial. **If a residual aliquot (<1.5 ml) is created, document the last four digits of the specimen 10-digit barcode and volume on the Biological Sample and Shipment Notification Form.**



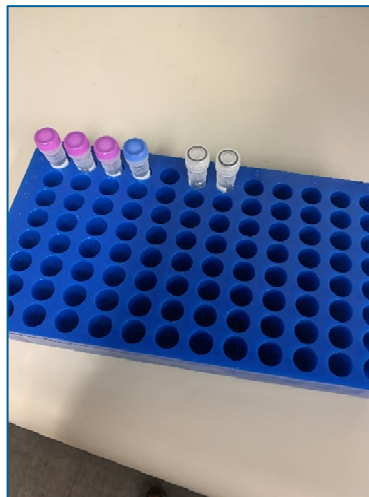
NOTE: When pipetting plasma from the plasma tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

11. 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 placed in freezer and storage temperature of freezer on Biological Sample Shipment and Notification Form.
12. 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 clean disposable transfer pipette. The buffy coat from each EDTA tube will be placed in a separate 2.0 ml cryovial with clear cap, and one of the two buffy coat aliquots will be submitted to NCRAD per participant, per visit. The buffy coat aliquot is expected to have a reddish color from the included RBCs. Be sure to place the buffy coat into the cryovial with the clear cap and "BUFFY COAT" label. Please place the buffy coat from only one blood tube in each cryovial.



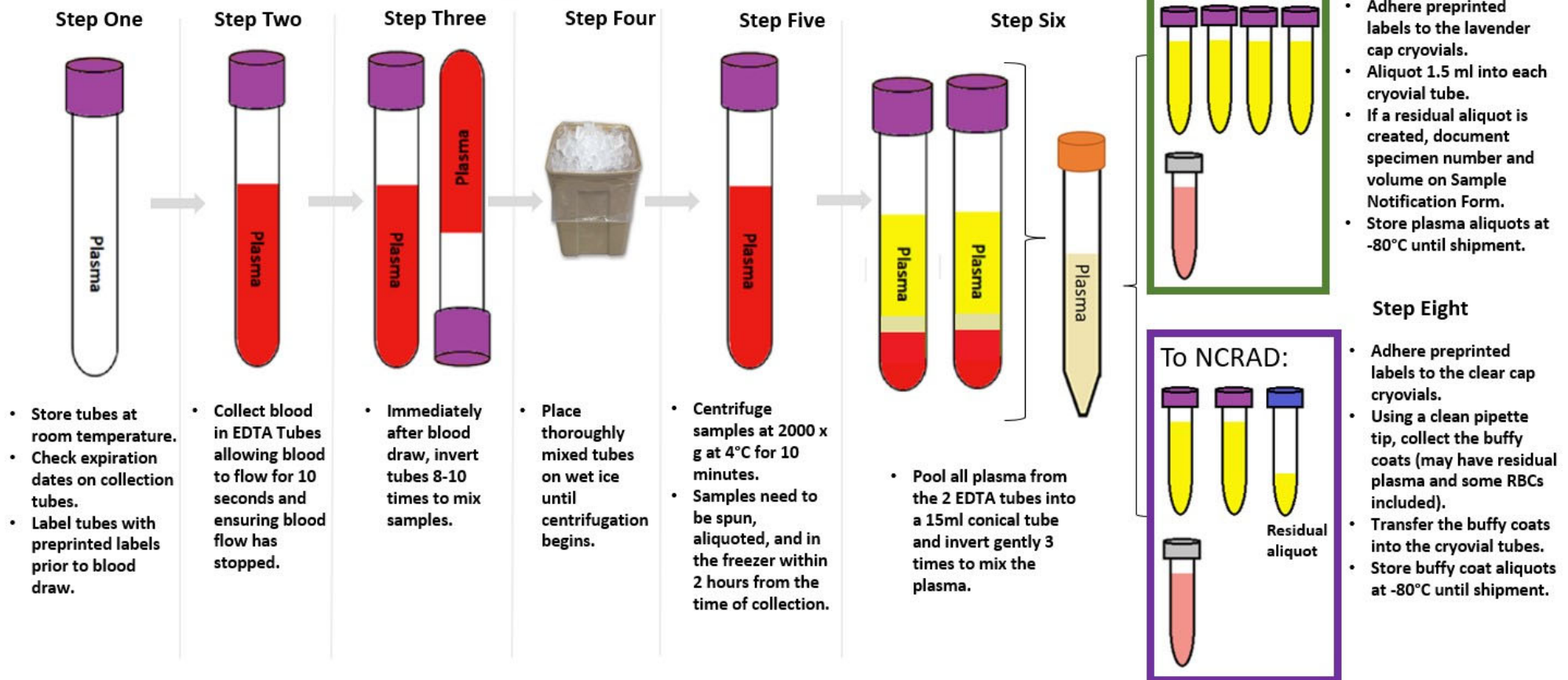


13. Dispose of collection tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.
14. Record the last four digits of the specimen 10-digit barcode and volumes of the EDTA tubes and corresponding buffy coat samples on the Biological Sample Shipment and Notification Form.
15. 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 placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.



Plasma Aliquots (up to 4 possible with 1 residual) and 2 Buffy Coats

Plasma and Buffy Coat Preparation (10ml Lavender-Top Tube x 2)



8.0 Packaging & Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your University, training and certification is available through the CITI training site (Course titled “Shipping and Transport of Regulated Biological Materials” at <https://www.citiprogram.org/>).

Sample Type	Study Visits Collecting Biospecimens	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes sent to NCRAD	Ship
Whole blood (Red Top Serum) for isolation of serum (Brown)	V01	1	N/A	0	N/A
Whole blood (Lavender-Top EDTA) for isolation of plasma & buffy coat (for DNA extraction) (Brown & NCRAD)	V01 - Brown	0 (samples being left at Brown)	1.5 ml plasma aliquots per 2.0 ml cryovial	0	N/A
	V01	4 (3 Lavender Cap, 1 Blue Cap Cryovial)	1.5 ml plasma aliquots per 2.0 ml cryovial	2	Frozen
	V01	2	750 ul buffy coat aliquot per 2.0 ml cryovial	1	Frozen

8.1 Frozen Packaging Instructions

IMPORTANT!
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.



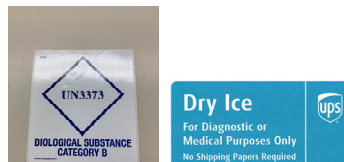
Large Frozen Shipper:
 ** 45 lbs of dry ice pellets
AND
 • Fits up to 8 x 25-cell cryoboxes

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 *****

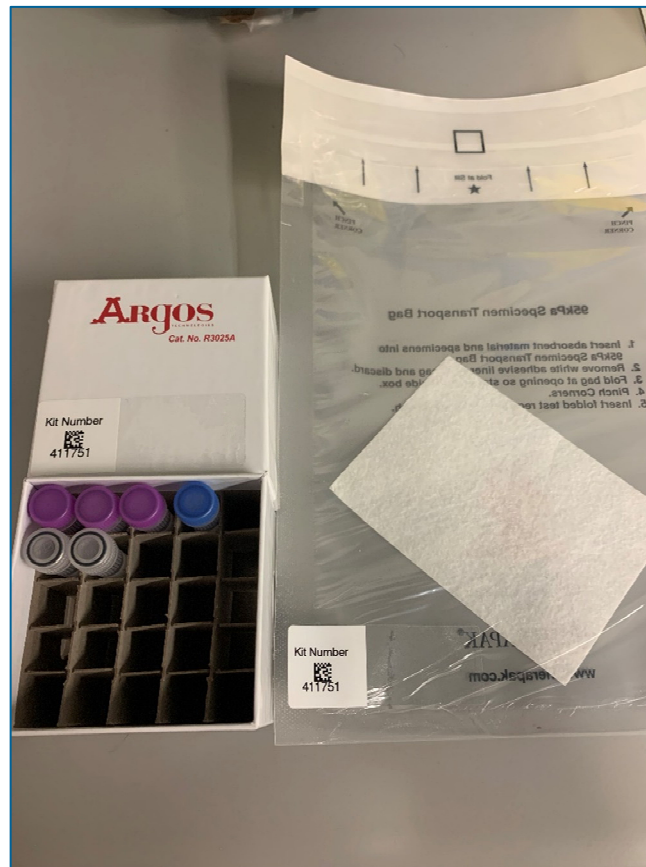
- The primary receptacle (frozen cryovials) must be leak-proof and must not contain more than 1L total.
- The secondary packaging (biohazard bag) must be leak-proof, and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (cryovial box containing the frozen cryovials) and the secondary packaging (biohazard bag). 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



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 of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu. Attach the following to the email:
 - a. Completed Biological Sample and Shipment Notification Form to the email notification. (See [Appendix A](#) for the associated NCRAD sample form)
 - b. If email is unavailable, please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.
3. Place all frozen labeled 1.5 ml aliquots of plasma and buffy coat in the 25-slot cryobox.

- a. Each cryobox holds up to 25 cryovials and there will be a maximum of 6 cryovials (4 plasma and 2 buffy coat) per blood draw (see following picture).

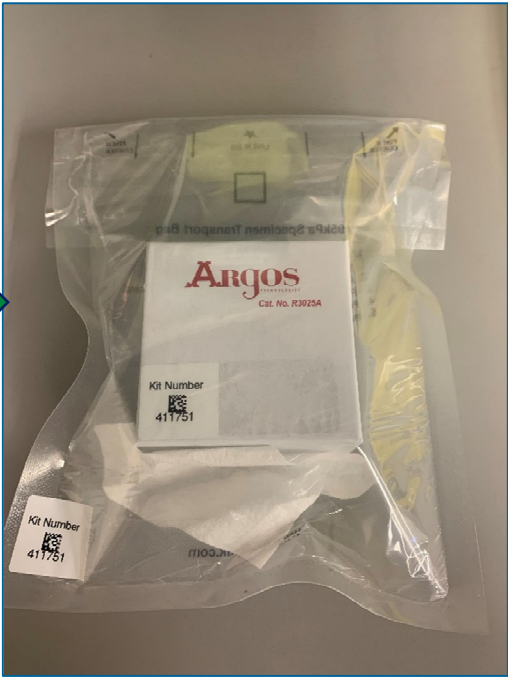
Blood kit Prepped for Shipping



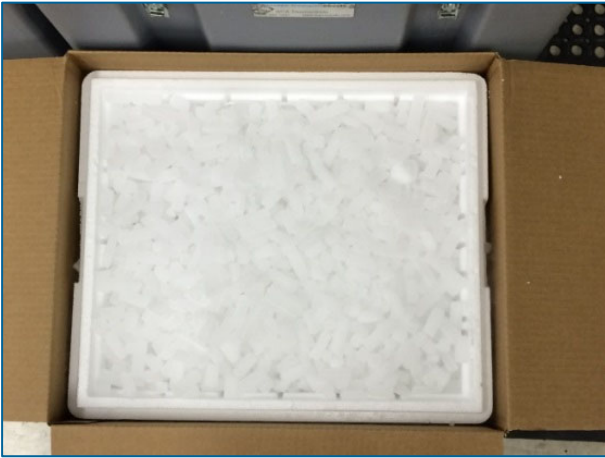
3. Label the outside of each cryobox with the kit number label (shown above). Please place the cryoboxes containing blood derivatives in one small biohazard bag. Label the outside of the small biohazard bag with a kit number label as well (shown above).
 - a. As the cryoboxes are placed in the small clear plastic biohazard bag, do NOT remove the absorbent material found in the bag. Seal according to the instructions on the bag. The kit number label should have been placed on each cardboard cryobox prior to inserting into the biohazard bag. A kit number label should also have been placed on the outside of the biohazard bag.

Cryobox and tubes placed in clear biohazard bags

Packaged Blood kit:
 One 25-count cryobox with plasma, buffy coats, and a residual aliquot (if applicable)



4. Place approximately 2-3 inches of pelleted dry ice in the bottom of the Styrofoam shipping container.
5. Place the biohazard bags into the provided Styrofoam-lined shipping container on top of the pelleted dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container. **A maximum of 8 cryoboxes may be sent in each shipper.**
6. Fully cover the biohazard bags containing the cryoboxes with approximately 2 inches of pelleted dry ice.



7. The inner Styrofoam shipping container must contain approximately 45 lbs (or 20kg) of pelleted dry ice. The pelleted dry ice should entirely fill the inner box and be placed on top of the biohazard bags to ensure the frozen state of the specimens.

8. Replace the lid on the Styrofoam carton. Place the completed Biological 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.
9. 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.
10. Apply all provided warning labels and UPS return airbill to the outside of package, taking care not to overlap labels.

IMPORTANT!

Ensure UPS address label is attached and UPS Dry Ice label is filled out, or UPS may reject or return your package.

11. Hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off. 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.
12. Specimens should be sent to the below address via **UPS Next Day Air**. Frozen shipments should be sent Monday through Wednesday to avoid shipping delays on Thursday or Friday. UPS does not replenish dry ice if shipments are delayed or held over during the weekend.

HALS at NCRAD
 Indiana University School of Medicine
 351 W. 10th St TK-217
 Indianapolis, IN 46202
 Phone: 1-800-526-2839

13. 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.

*****Important Note*****

For frozen shipments, include no more than eight cryovial boxes (separated by patient within biohazard bags) per shipping container in order to have room for a sufficient amount of pelleted dry ice to keep samples frozen up to 24 hours.

The labeled, processed, aliquoted, and frozen cryovials of plasma and buffy coat must be shipped to NCRAD as outlined above.

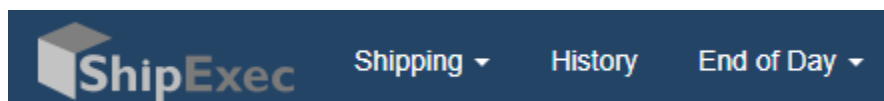
**SHIP ALL FROZEN SAMPLES MONDAY - WEDNESDAY ONLY!
BE AWARE OF HOLIDAYS!!
BE AWARE OF INCIPIENT INCLEMENT WEATHER THAT MAY DELAY
SHIPMENT/DELIVERY OF SAMPLES**

Remember to complete the Biological Sample and Shipment Notification (Appendix A), include a copy in your shipment **AND** notify the NCRAD Study Coordinator by email at alzstudy@iu.edu (include UPS tracking number in email) **IN ADVANCE** to confirm the shipment.

In addition to tracking and reconciliation of samples, the condition and number 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 samples are packed with enough pelleted dry ice to avoid thawing in the shipment process.

8.2 Frozen Shipping Instructions

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



3. Click on the magnifying glass icon in the “Ship From” section to search for your shipping address.

Ship From

Company	
Contact	
Address 1	
Address 2	
Address 3	
City	
State/Province	
Postal Code	
Country/Territory	▼
Phone	

- 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.
4. 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.
 5. Enter Package Information
 - a. Frozen shipments
 - i. Enter the total weight of your package in the “Weight” field.
 - ii. Enter the 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 and need to reenter these values.
 - b. Click Ship in the bottom right of the page when complete.
 6. Print the airbill that is automatically downloaded.
 - a. To reprint airbill, click History at the top left of the page.
 - b. Click Detailed Report from the dropdown menu on the right side of the page.
 - c. Enter tracking number if known. Otherwise, search by ship date. Click Search.
 - d. Click print icon on right side of the tracking number line.

7. A UPS Pickup is automatically scheduled at the address you are shipping from, and the pickup is charged to NCRAD.
 - a. If shipment occurs too late in the day for an automatic UPS pickup, you will receive an email stating that the pickup could not be scheduled, and you will need to make other arrangements.

9.0 Data Queries and Reconciliation

The laboratory worksheets must be completed on the day that samples are collected, since they capture information related to the details of the sample collection and processing. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

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

- Missing samples
- 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 Biological Sample and Shipment Notification Form and logged at NCRAD.
- Samples that are frozen and stored longer than one quarter at the site
- Use of an incorrect Biological Sample and Shipment Notification Form

10.0 Appendices

[Appendix A: Blood Sample and Shipment Notification Form](#)

[Appendix B: Video List](#)

[Appendix C: GUID Demographics Form](#)

[Appendix D: Rate of Centrifugation Worksheet](#)



Appendix A: Blood Sample and Shipment Notification Form

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

To: Kelley Faber	Email: alzstudy@iu.edu	Phone: 1-800-526-2839
From: _____	UPS tracking #: _____	
Phone: _____	Email: _____	Site: Brown University
Study: HALS (Healthy Aging Across the Lifespan)		
PTID: _____	Visit: V01	KIT BARCODE
GUID: _____		
Sex: <input type="checkbox"/> M <input type="checkbox"/> F	Year of Birth: _____	
Blood Collection:		
Date of Draw: _____ [MMDDYY]		Time of Draw: _____ [HHMM]
Date participant last ate: _____ [MMDDYY]		Time participant last ate: _____ [HHMM]
Blood Processing:		
Plasma & Buffy Coat (Lavender-Top) Tube (10 mL)		
Time spin started:	_____ [HHMM]	
Duration of centrifugation:	_____ Minutes	
Temp of centrifuge: _____ °C	Rate of centrifuge: _____ x g	
Time aliquoted:	_____ [HHMM]	
Number of 1.5 mL plasma aliquots created (lavender cap, up to 6):	_____	
If applicable, volume of residual plasma aliquot (less than 1.5 mL in blue cap):	_____ mL	
If applicable, specimen number of residual plasma aliquot (last four digits):	_____	
Buffy coat #1 last four digits of specimen number: _____		
Buffy coat #1 volume: _____ mL	Original blood volume drawn: _____ mL	
Buffy coat #2 last four digits of specimen number: _____		
Buffy coat #2 volume: _____ mL	Original blood volume drawn: _____ mL	
Time plasma and buffy coat aliquots frozen:	_____ [HHMM]	
Notes:		

Appendix B: Video List

- NCRAD offers training videos that are available to assist you with the specimen processing, aliquoting, and shipping processes.
 - Frozen Shipping
 - Ambient Shipping
 - Plasma and Buffy Coat Processing and Aliquoting
 - HALS MOP Training



Appendix C: 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. Only send the GUID to NCRAD.

1. Complete 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 D: Rate of Centrifugation 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).

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

alzstudy@iu.edu