BenfoTeam Manual of Procedures Update: Version 04.17.2025

Section	Change
Section 9.1.1	Adjusted language highlighting requirement for Blood and Sample Shipment Notification Form to be e-mailed prior to shipping specimens
Section 9.1.1	Adjusted language highlighting requirement for Blood and Sample Shipment Notification Form to be printed and included in physical shipping box.
Section 6.1	Included important note instructing use of screening kits for screening visit collections and visit kits for visit collection accordingly
Section 3.3	Winter break holiday added
Section 7.1.2	Label Abbreviation Summary table added
Section 7.1.	Updated label diagrams
Section 7.1	Added cryovial label language
Section 7.1	Updated label format
Section 5.2.2	Updated consent language URL
Section 3.3	Updated NCRAD Holiday Closure URL
Appendix C, Appendix D	Hyphen removed from Participant ID format
Appendix C	Residual plasma volume changed from less than 1.5 ml to less than 0.5 ml
Appendix C, Appendix D	“Participant ID” and protocol number added
Appendix C	Corrected plasma aliquot size volume from 1.5ml to 1.0 ml and 0.5 ml
Section 5.2.2	Expanded “All Visits” to list specific visit timepoints in “Drawn at” column of Biofluid Collection Chart
Throughout	Alternate text added to tables throughout.
Section 3.1	Added new URL for BenfoTeam study webpage on NCRAD website
Appendix C	Whole blood cap color changed from “clear” to “yellow”
Appendix C	Washed RBC cap color changed from “clear” to “red”

A Seamless Phase 2A-Phase 2B Randomized Double-Blind Placebo-Controlled Trial to Evaluate the Safety and Efficacy of Benfotiamine in Patients with Early Alzheimer's Disease (BenfoTeam)

in collaboration with the

National Centralized Repository for Alzheimer's Disease and Related Dementias

Biospecimen Collection, Processing, and Shipment Manual of Procedures

Version 04.17.2025

TABLE OF CONTENTS

1.0 Abbreviations

AD	Alzheimer's Disease
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
GUID	Globally Unique Identifier
IATA	International Air Transport Association
NACC	National Alzheimer's Coordinating Center
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PHI	Protected Health Information
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
ETKAC	Erythrocyte Tranketolase Activity Coeffiecient

2.0 Purpose

The collection of biofluids is an important part of A Seamless Phase 2A-Phase 2B Randomized Double-Blind Placebo-Controlled Trial to Evaluate the Safety and Efficacy of Benfotiamine in Patients with Early Alzheimer's Disease (BenfoTeam) Study. 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 BenfoTeam study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

Sites will collect and send the following samples to NCRAD:

- Plasma
- Buffy Coat (DNA Extraction)
- Cerebrospinal Fluid (CSF)
- Washed RBCs
- Whole Blood

This manual includes instructions for collection of blood and CSF, 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 BenfoTeam protocol.

3.0 NCRAD 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

Erin Delaney, BS, CCRP, Clinical Research Coordinator
Phone: 317-274-1221
Email: eridelan@iu.edu

General NCRAD Contact Information
Phone: 1-800-526-2839
Email: alzstudy@iu.edu
Website: www.ncrad.org
BenfoTeam Webpage: www.ncrad.org/coordinate-studies/benfoteam

Sample Shipment Mailing Address
BenfoTeam at NCRAD
Indiana University School of Medicine
351 W. 10th St. TK-342
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 samples, please refer to Section 9.0 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

Date	Holiday
4th Thursday in November	Thanksgiving
4th Friday in November	Friday after Thanksgiving
December 25	Christmas Day
December 26-31	Winter Break

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/contact/holiday-closures **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 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 BenfoTeam 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 ≥ 2000 x g with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:

- Pelleted dry ice (approximately 45 lbs per shipment)

Samples are to be submitted according to the shipping methods outlined in Section 9.0. Guidelines for the processing, storage location, and timing of sample collection are listed in the following tables.

5.2 Biospecimens Sent to NCRAD

5.2.1 Biofluid Collection Schedule

Timepoint	Pre-Dose				Post Dose				Optional
	Whole Blood	Plasma	Buffy Coat	Washed RBC	Whole Blood	Plasma	Buffy Coat	Washed RBC	CSF
Screening		X	X						
Baseline	X	X	X	X	X	X	X	X	X*
Week 8	X	X	X	X	X	X	X	X	
Week 72	X	X	X	X					X*
Early Termination	X	X	X	X					

***** CSF samples should be collected at approximately the same time of day at each collection timepoint. Visit windows for optional lumbar puncture at baseline for CSF are: up to 42 days prior to first dose of IP and within 7 days of the week 72 study visit.

Screening visits **must** be recorded in **www.kits.iu.edu/BENFOScreening**. As soon as a screening visit is scheduled, report the following information in this form: PTID, Kit Number, Date of Visit, Date of Shipment. This ensures for the most efficient inclusion/exclusion results.

If a patient is terminating early, they do not need to undergo lumbar puncture.

Sites are required to contact the study partner the evening prior to the Week 8 and Week 72 Visits to remind study partners not to administer the Investigational Product (IP) at home.

- Pre-dose: prior to morning IP intake
- Post-dose: approximately 2 hours (+/- 30 minutes) after the morning IP intake

5.2.2 Biofluid Collection Chart

Collection Tube	Drawn at	Processed Specimen Type	Number of Aliquots	Volume of Aliquots	Cap Color
3 x 10 ml Whole-Blood Purple-Top EDTA Tubes	Screening, Baseline, Week 8, Week 72, Early Termination	Plasma	5	1.0 ml	Purple
		Plasma	20	0.5 ml	Purple
		Residual Plasma	1	<0.5 ml	Blue
		Buffy Coat	3	1.0 ml	Clear
	Baseline, Week 8, Week 72, Early Termination	Whole Blood	1	1.0 ml	Yellow
		Washed RBCs	1	1.5 ml	Red
1 x 20 ml CSF Blue-Top Conical Tube	Baseline & Week 72 (Optional)	Local Lab CSF	1	1.0 -2.0 ml	Yellow
		Processed CSF	11	1.5 ml	Orange
		Residual CSF	1	<1.5 ml	Blue

The 10 ml EDTA tubes are processed locally into plasma and buffy coat fractions; they are then aliquoted, frozen at the study site. For qualifying study visits, whole-blood and washed RBCs will also be processed locally, aliquoted, and frozen at study sites. If collected, CSF will also be aliquoted and frozen at the study site. All processed aliquots will be packaged in frozen shipments and sent to NCRAD. 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 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.

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, the Blood Supplemental Supply Kit, the Frozen Shipment Kit; 2) CSF collection kits including Lumbar Puncture (LP) trays, the CSF Supplemental Supply Kit; and 3) 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, LP trays (when applicable), boxes for plasma, buffy coat, whole-blood, washed RBCs, and CSF aliquots, as well as shipping materials to send biospecimens to NCRAD. Kit number labels, site and Participant ID 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 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.

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. For visits that involve a pre- and post-dose collection, one kit should be used for the pre-dose collection and one kit should be used for the post-dose collection. 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. Ensure no tubes have past expiration date before use.* Verify which specimens will be collected during specified visits prior to ordering visit-specific collection kits. See charts in Section 5.2.1.

Important Note: Do not swap kits designed for screening collection with visit collection and vice versa.

BenfoTeam Blood Collection Kits

Quantity	Screening Blood Kit
3	EDTA tube, 10ml
1	Cryovial, 2ml, blue cap, assembled
3	Cryovial, 2ml, clear cap, assembled
25	Cryovial, 2ml, purple cap, assembled
31	Collection Tube/Aliquot Labels
4	Kit Number Labels
4	Participant ID Labels
5	Disposable pipet (3ml)
1	50 ml conical tube
2	Cardboard cryobox, 25 slot
1	Resealable small poly bag (4" x 6")
1	Resealable bag

Quantity	Baseline, Week 8, Week 72, Early Termination Blood Kit
3	EDTA tube, 10ml
1	Cryovial, 2ml, blue cap, assembled
1	Cryovial, 2ml, yellow cap, assembled
3	Cryovial, 2ml, clear cap, assembled
25	Cryovial, 2ml, purple cap, assembled
1	Cryovial, 2ml, red cap, assembled
34	Collection Tube/Aliquot Labels
4	Kit Number Labels
4	Participant ID Labels
5	Disposable pipet (3ml)
1	50 ml conical tube
1	Centrifuge tube, 15 ml
2	Cardboard cryobox, 25 slot
1	Resealable small poly bag (4" x 6")
1	Resealable bag

BenfoTeam Supplemental Supply Kit

Quantity	Supplemental Supply Kit
1	Normal Saline – 500ml bottle
6	EDTA tube, 10ml
4	Cryovial, 2ml, blue cap, assembled
50	Cryovial, 2ml, purple cap, assembled
12	Disposable pipet (3ml)
2	Conical tube, 50 ml
4	Resealable bag
26	Cryovial, 2ml, clear cap, assembled
4	Cryovial, 2mL, yellow cap, assembled
2	Cryovial, 2ml, red cap, assembled
4	15ml conical, individually wrapped
2	Cardboard cryobox, 25 slot
5	Participant ID Labels
5	Dry Ice label
5	UN3373 sticker
5	Fragile labels
5	Biohazard bag w/ absorbent sheet

BenfoTeam LP Kits

Quantity	LP Kit Components
1	Sprotte needle, 22 X 3.5” (90mm)
1	Introducer needle, 1 mm x 30 mm
1	Hypodermic needle, 22 gauge x 1.5”
1	Plastic syringe, (3 ml, luer lock) with 25G x 5/8” needle attached
4	Polypropylene syringe (5 ml, luer lock)
1	Needle stick pad

Quantity	LP Kit Components
1	Adhesive bandage
1	Drape, fenestrated, 2 tabs, paper, 18” x 26”
2	Towel, 13.5” x 18”
6	Gauze pad, 2” x 2”
3	Sponge stick applicator
2	Lidocaine 1%, 5 ml
1	Povidone-Iodine Topical Solution, 0.75 oz

BenfoTeam CSF Collection Kits

Quantity	CSF Kit Components
10	Cryovial tube (2.0 ml) with clear cap
1	Cryovial tube (2.0 ml) with yellow cap
1	Cryovial tube (2.0 ml) with blue cap
2	15 ml conical tube, individually wrapped
1	50 ml Conical tube, individually wrapped
1	Disposable pipet (3.0 ml)
13	Collection/Aliquot Tube Labels
1	Participant ID Label
1	Kit Number Label
1	Resealable bag
1	Resealable small poly bag (4”x 6”)
1	Cardboard cryobox, 25 slot

BenfoTeam Frozen Shipping Kits

Quantity	SCREENING (Individual) Frozen Shipping Kit Components
1	Dry Ice label
1	UN3373 sticker
1	Fragile label
1	Small frozen shipper/Small brain box
3	Biohazard bag w/ absorbent sheet
1	Resealable bag
1	Shipping Pouch

Quantity	STUDY VISITS (Batch) Frozen Shipping Kit Components
1	Dry Ice label
1	UN3373 sticker

Quantity	STUDY VISITS (Batch) Frozen Shipping Kit Components
1	Fragile labels
1	Medium frozen shipper/Large brain box
8	Biohazard bag w/ absorbent sheet
1	Resealable bag
1	Shipping Pouch

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module
By Request	EDTA tube, 10ml
By Request	Cryovial, 2ml, blue cap, assembled
By Request	Cryovial, 2ml, purple cap, assembled
By Request	Disposable pipet (3ml)
By Request	Centrifuge tube, 50 ml
By Request	Resealable bag
By Request	Cryovial, 2ml, clear cap, assembled
By Request	Cryovial, 2mL, yellow cap, assembled
By Request	Cryovial, 2ml, red cap, assembled
By Request	15ml conical, individually wrapped
By Request	UPS Airbill Sleeve
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	Warning label packet (UN3373 label, Biohazard label, and Dry ice shipping label)
By Request	UN3373 label
By Request	Biohazard label
By Request	Dry ice shipping label
By Request	UPS Airbill Sleeve
By Request	Participant ID 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.

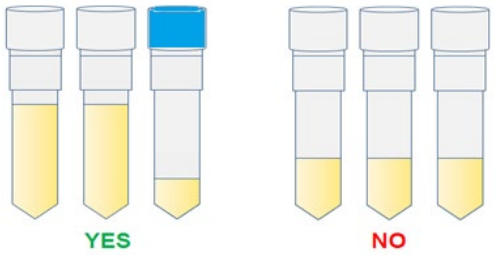
Please go to: **www.kits.iu.edu/BENFO** to request additional kits and follow the prompts to request the desired supplies.

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

6.3 Filling Cryovials (Plasma and CSF)

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 cryovial should be filled to the assigned volume with the respective biological material 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 that sample.

Aliquot the remaining biologic material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 3.3 ml of sample is obtained, you should fill 6 cryovial tubes each with 0.5 ml, and one additional cryovial tube with the remaining 0.3 ml of sample.



Please note: It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume (anything under 0.5 ml for plasma and CSF). Please record the specimen number and volume of the residual aliquot on the Biological Sample and Notification Form.

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
Purple	Plasma
Clear	Buffy Coat
Blue	Residual sample (Plasma and/or CSF)
Yellow	Whole Blood
Red	Washed RBCs
Yellow	Local Lab CSF
Clear	Processed CSF

7.0 Blood Collection and Processing Procedures

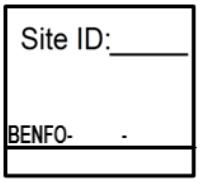
7.1 Labeling Samples

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 PTID Label
3. Collection Tube Label
4. Aliquot Cryovial Label

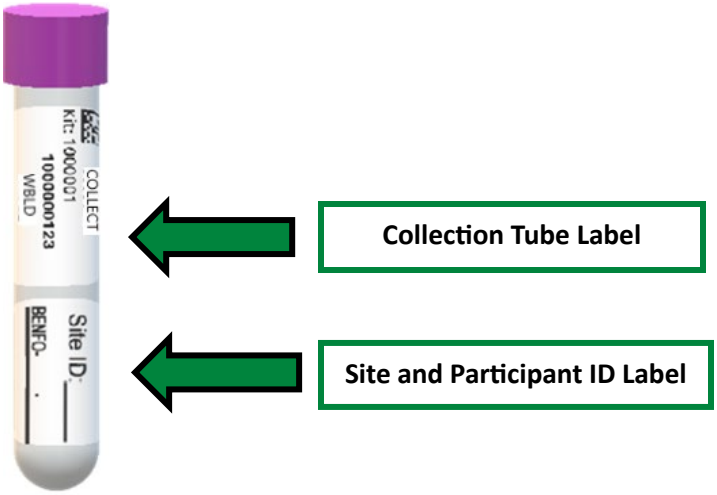
Kit Number Labels tie together all specimens collected from one participant at one visit. They should be placed on each cryobox and in the designated location on the Blood Sample and Shipment Notification Forms.



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

Place one **Collection Tube Label** on each EDTA collection tube.

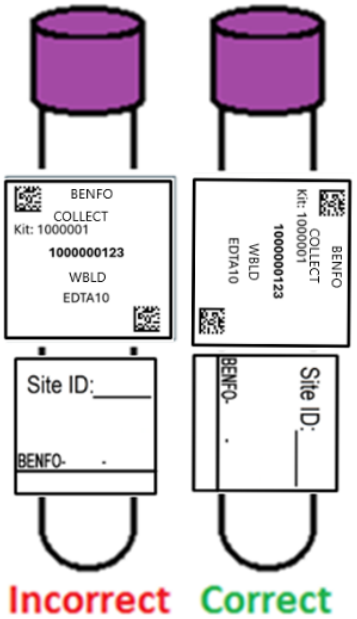
Place one **Aliquot Label** on each aliquot cryovial with correct specimen type.



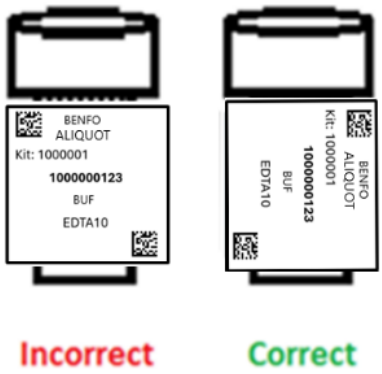
Labeled EDTA (Purple-Top) Blood Collection Tube

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

Each aliquot cryovial will contain one label: the aliquot label is specimen specific and contains a unique barcode and number. Be sure that the specimen type, aliquot cap color, and specimen label correspond according to protocol.



Collection Tube Label Diagram



Aliquot Label Diagram

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 Participant ID Labels on the EDTA (purple-top) tubes **BEFORE** sample collection. These labels are placed on collection tubes in addition to the Collection Tube and Aliquot Label.
- The Collection Tube and Aliquot 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.

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.

7.1.2 Label Abbreviation Summary

Specimen Type	Specimen Abbreviation	Container/Cap Color
Whole Blood	WBLD	EDTA 10 ml/purple cap
Whole Blood	WBLD	Cryovial 2ml/yellow cap
Plasma	PLA	Cryovial 2ml/purple cap
Buffy Coat	BUF	Cryovial 2ml/clear cap
Washed RBC	RBC	Cryovial 2ml/red cap
Cerebrospinal Fluid	CSF	Cryovial 2ml (see table 5.2.2)

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

1. Report Screening Visit information at www.kits.iu.edu/BENFOScreening.

2. Store empty EDTA tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use. Ensure EDTA tube is used prior to expiration date.

3. Set centrifuge to 4°C to pre-chill before use.

4. Place completed site and Participant ID 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 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.

5. 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 final tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

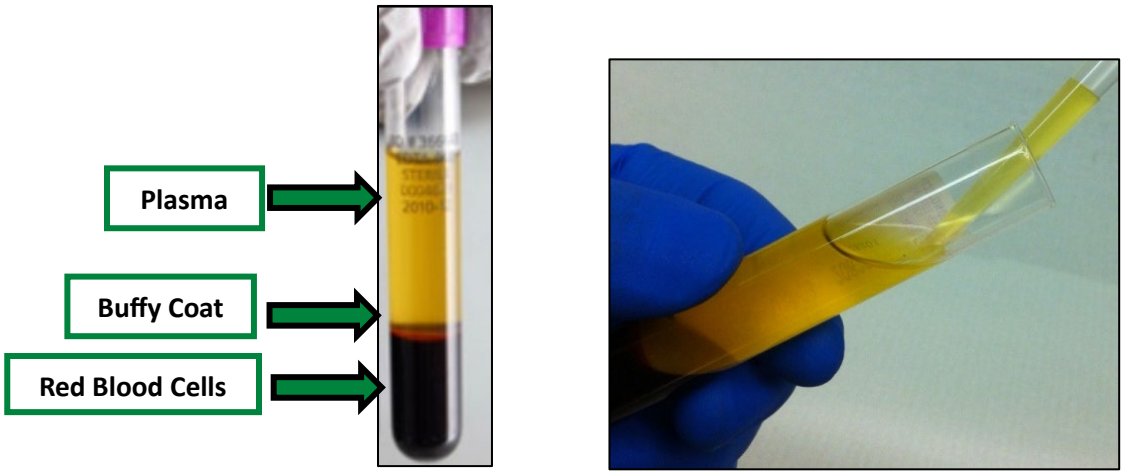
6. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
   a. If complications arise during the blood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

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

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

9. Centrifuge balanced tubes for 10 minutes at 2000 x g at 4°C. **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 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.

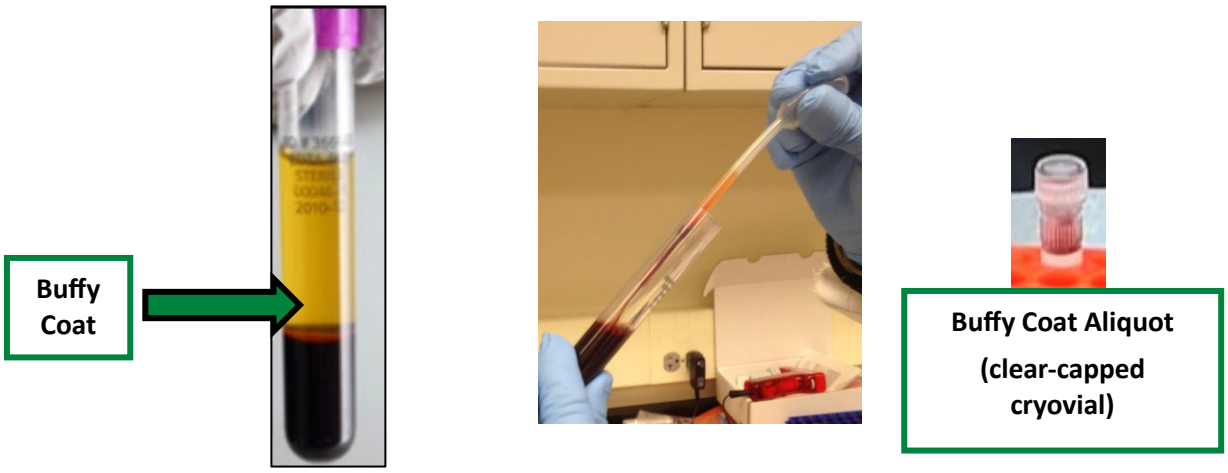
10. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall being careful not to agitate the packed red blood cells at the bottom of the collection tube.



11. Each EDTA tube should yield, on average, 4-5 ml of plasma. Transfer plasma from all EDTA tubes into the 50 ml conical tube (for 30 ml collections) 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.** Aliquot 1.0 ml of plasma into 5 cryovials and then aliquot 0.5 ml into 20 cryovials . Be sure to only place **plasma** in cryovials with purple caps and labeled with **PLASMA** labels**.** Place residual plasma (<0.5 ml) in the blue-capped cryovial. **If a residual aliquot (<0.5 ml) is created, document the specimen number and volume on the Biological Sample and Shipment 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 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.

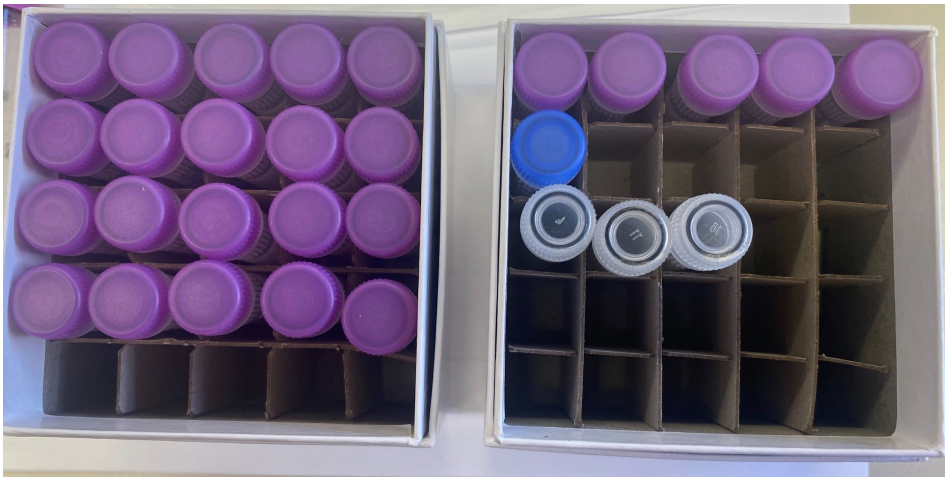
Repeat this step for the second and third EDTA tubes (when collecting 30ml total), placing these buffy coats into the second and third clear-capped cryovials.



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

14. **For qualifying study visits: keep collection tube containing red blood cell pellet for further processing and aliquoting.** If study visit only requires plasma and buffy coat collection: dispose of collection tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.

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 frozen and storage temperature of freezer on Biological Sample and Shipment Notification Form (Appendix C).



For Screening Visit

Plasma Aliquots: will consists of 5 purple capped cryovials containing 1.0 ml and 20 purple capped cryovials containing 0.5 ml. Any residual plasma (<0.5 ml) will be place in 1 blue capped cryovial.

Buffy Coat Aliquots: will consist of 3 clear capped cryovials containing ~1.0 ml of buffy coat collected from three corresponding 10 ml EDTA collection tubes

Screening Visit

Processing ONLY Plasma & Buffy Coat from EDTA Purple-Top Tube (3x10ml)

Step One

- ❑ Store tubes at room temperature
- ❑ Label each tube with appropriate pre-printed labels prior to blood draw

Step Two

- ❑ Collect 10 ml of blood into each EDTA tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three

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

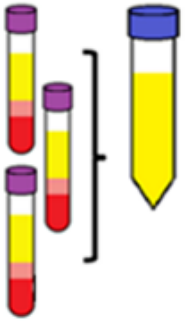
Step Four

- ❑ Placed thoroughly mixed tubes on wet ice until centrifuge begins

Step Five

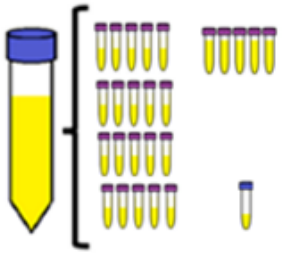
- ❑ Replace caps to all 3 EDTA's prior to centrifuge.
- ❑ Centrifuge samples at 2000 x g for 10 minutes at 4° C

Step Six



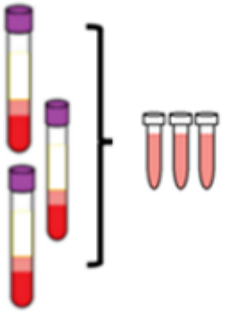
- ❑ Pool all plasma from 3 EDTA tubes into a 50 ml Conical tube
- ❑ Gently invert conical tube 3 times to mix the plasma

Step Seven



- ❑ Label purple top cryovials with "PLASMA" labels.
- ❑ Aliquot 1.0 ml of plasma from conical tube into 5 purple top cryovials
- ❑ Aliquot 0.5 ml of plasma from conical tube into 20 purple top cryovials
- ❑ Aliquot any residual plasma of <0.5 ml into the blue top cryovial
- ❑ Document specimen number and volume of residual plasma on Sample form.
- ❑ Store all plasma aliquots upright at -80° C until shipment to NCRAD

Step Eight



- ❑ Label clear top cryovials with "BUFFY COAT" labels
- ❑ Using a clean transfer pipette, collect the buffy coat from EDTA tubes (may have some residual plasma and some RBC's included)
- ❑ Transfer the buffy coat from each EDTA tube into its own cryovial
- ❑ Store all buffy coat aliquots upright at -80° C until shipment to NCRAD
- ***Note: spin, aliquot and freeze all samples within 2 hours of collection**

7.3 Baseline, Week 8, Week 72, and Early Termination: Whole Blood Collection with 10 ml EDTA (Purple-Top) Tube for Whole-Blood, Plasma, Buffy Coat and Washed RBCs

1. Store empty EDTA tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use. Ensure EDTA tube is used prior to expiration date.

2. Set centrifuge to 4°C to pre-chill before use.

3. Place completed site and Participant ID Label and preprinted Collection Tube Label on the purple-top EDTA tubes. Place preprinted **PLASMA** Aliquot Labels on the 2 ml cryovials with purple 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. Place preprinted **WHOLE BLOOD** Aliquot Label on the 2 ml cryovial with yellow cap. Place preprinted **WASHED RBC** Aliquot Label on the 2 ml cryovial with red cap.

4. 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 final 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 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.

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.

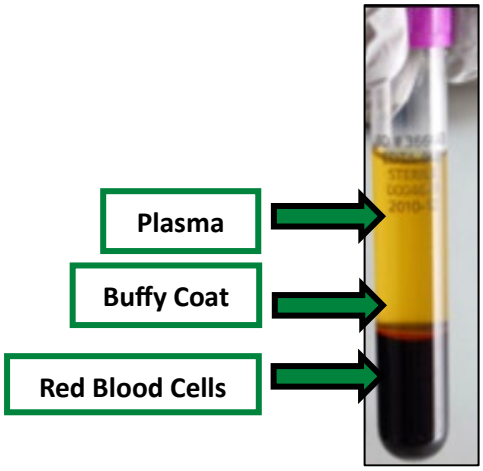
8. **Prior to processing for plasma and buffy coat,** aliquot 1.0 ml of whole blood from **ONE** of the EDTA blood collections into yellow capped cryovial labeled "WHOLE-BLOOD".

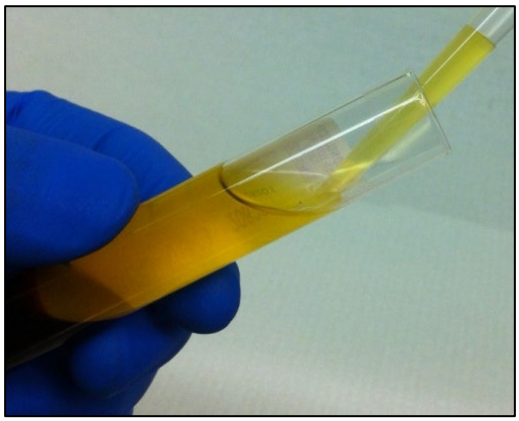
9. Place the labeled WHOLE-BLOOD cryovial on pelleted dry ice. Transfer to -80°C Freezer when possible. Store all samples at -80°C in freezer until shipped to NCRAD on pelleted dry ice. Record time aliquots frozen and storage temperature of freezer on Biological Sample Shipment and Notification Form (Appendix C).

10. Ensure all purple tops have been replaced on EDTA tubes before centrifuge.

11. Centrifuge balanced tubes for 10 minutes at 2000 x g at 4°C. **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 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.

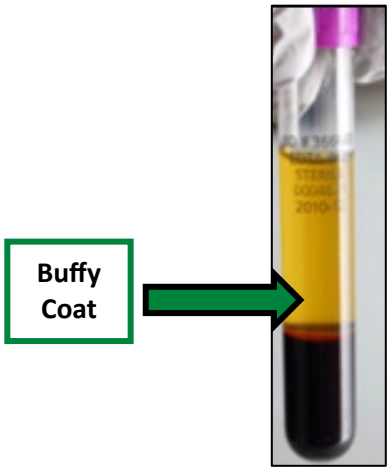
12. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall being careful not to agitate the packed red blood cells at the bottom of the collection tube.

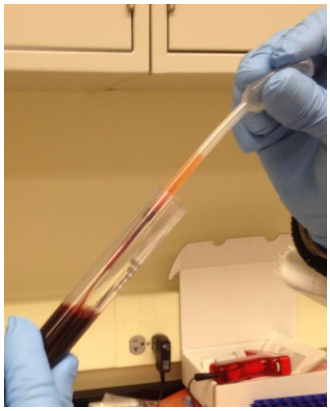




13. Each EDTA tube should yield, on average, 4-5 ml of plasma. *Transfer plasma from all EDTA tubes into the 50 ml conical tube* and gently invert 3 times. Aliquot 1.0 ml of plasma into 5 purple capped cryovials and then aliquot 0.5 ml into 20 purple capped cryovials. Be sure to only place plasma in cryovials with purple caps that have been labeled with **PLASMA** labels. Place residual plasma (<0.5 ml) in the blue-capped cryovial. If a residual aliquot (<0.5 ml) is created, document the specimen number and volume on the Biological Sample and Shipment Notification Form.

14. 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 labeled with **BUFFY COAT** labels.





Buffy Coat Aliquot

(clear-capped cryovial)

15. To the remaining RBCs in **ONE** of the EDTA tubes; add normal saline (0.9% w/v NaCl solution) to equal the approximate volume of whole blood previously collected during Step 5; (~10 ml).

16. Gently invert the tube 10 times to mix.

17. Centrifuge EDTA tube containing saline and RBCs at 2000 x g for **5 minutes** at 4°C.

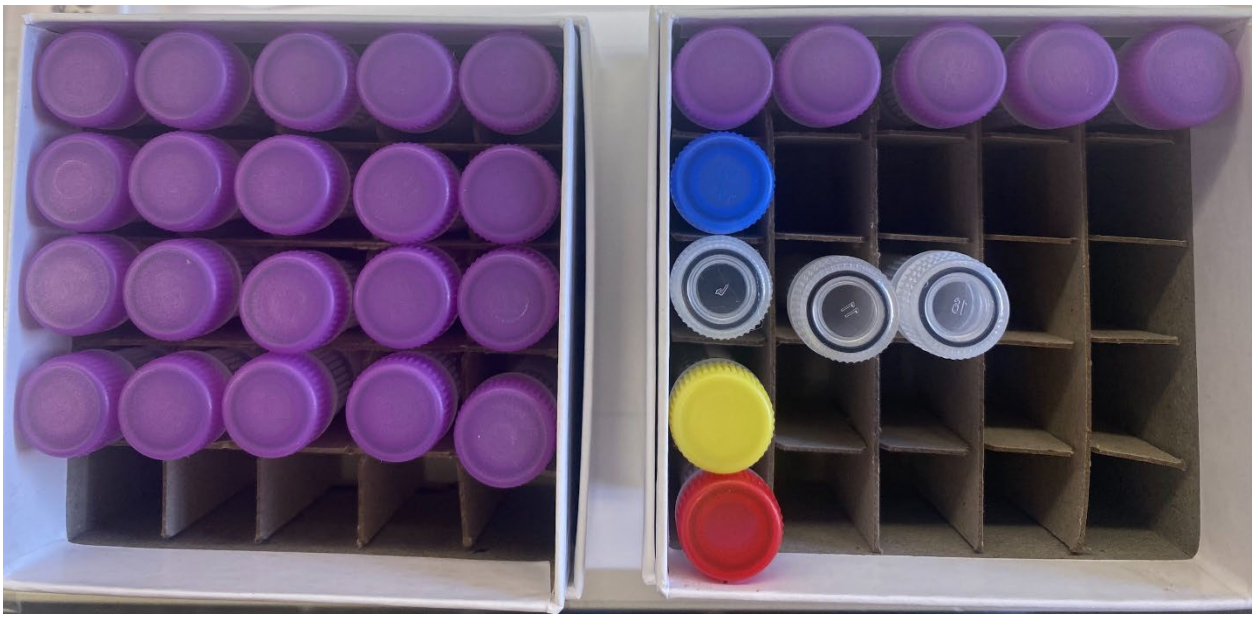
18. Using a clean transfer pipette; remove and discard the saline supernatant.

19. Repeat steps 15-18 once more for a total of 2 washes or until the supernatant is clear (not red). Ensure complete removal of the final saline supernatant. *Note: It is better to remove a mm of RBCs with the saline rather than leaving any saline in the sample.

20. Aliquot 1.5 ml of washed RBCs to one red capped cryovial labeled “RBC”.

21. Place the labeled WASHED RBC cryovial on pelleted dry ice. Transfer to -80°C Freezer when possible.

22. Place all 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 Biological Sample and Shipment Notification Form.



For Baseline, Week 8, Week 72, and Early Termination Visit

Whole Blood Aliquot: will consist of 1 yellow capped cryovial containing 1.0 ml

Plasma Aliquots: will consists of 5 purple capped cryovials containing 1.0 ml and 20 purple capped cryovials containing 0.5 ml. Any residual plasma (<0.5 ml) will be place in 1 blue capped cryovial.

Buffy Coat Aliquots: will consist of 3 clear capped cryovials containing ~1.0 ml of buffy coat collected from three corresponding 10 ml EDTA collection tubes

Washed RBC Aliquot: will consist of 1 red capped cryovial containing 1.5 ml of washed RBCs

Baseline, Week 8, Week 72 and Early Termination Visit

Processing Whole-Blood, Plasma, Buffy Coat and Washed RBCs from EDTA Purple-Top Tube (3x10ml)

Step One

- ❑ Store tubes at room temperature
- ❑ Label each tube with appropriate pre-printed labels prior to blood draw

Step Two

- ❑ Collect 10 ml of blood into each EDTA tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

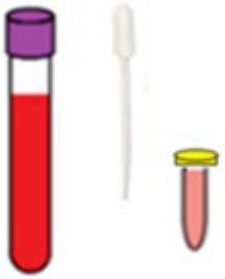
Step Three

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

Step Four

- ❑ Placed thoroughly mixed tubes on wet ice until centrifuge begins

Step Five



- ❑ Prior to processing for plasma and buffy coat, aliquot 1.0 ml of whole blood from **ONE** of the EDTA blood collections into one yellow capped cryovial labeled "WHOLE-BLOOD".
- ❑ Store whole blood aliquot at -80° C freezer until shipment to NCRAD

Step Six

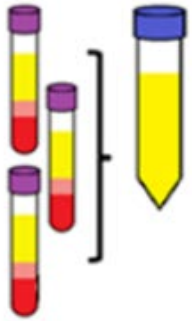
- ❑ Replace caps to all 3 EDTA's prior to centrifuge.
- ❑ Centrifuge samples at 2000 x g for 10 minutes at 4° C

Continue for Next Steps

Baseline, Week 8, Week 72, and Early Termination (continued)

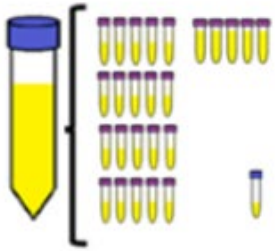
Processing Whole-Blood, Plasma, Buffy Coat and Washed RBCs from EDTA Purple-Top Tube (3x10ml)

Step Seven



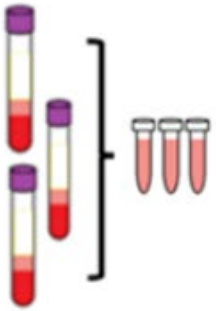
- ❑ Pool all plasma from 3 EDTA tubes into a 50 ml Conical tube
- ❑ Gently invert conical tube 3 times to mix the plasma

Step Eight



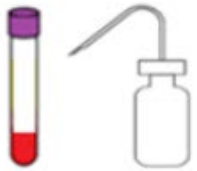
- ❑ Label purple top cryovials with "PLASMA" labels.
- ❑ Aliquot 1.0 ml of plasma from conical tube into 5 purple top cryovials
- ❑ Aliquot 0.5 ml of plasma from conical tube into 20 purple top cryovials
- ❑ Aliquot any residual plasma of <0.5 ml into the blue top cryovial
- ❑ Document specimen number and volume of residual plasma on Sample form.
- ❑ Store all plasma aliquots upright at -80° C until shipment to NCRAD

Step Nine



- ❑ Label clear top cryovials with "BUFFY COAT" labels
- ❑ Using a clean transfer pipette, collect the buffy coat from EDTA tubes (may have some residual plasma and some RBC's included)
- ❑ Transfer the buffy coat from each EDTA tube into its own cryovial
- ❑ Store all buffy coat aliquots upright at -80° C until shipment to NCRAD

Step Ten



- ❑ To the remaining RBCs in <u>ONE</u> of the EDTA tubes; add normal saline (0.9% w/v NaCl solution) to equal the approximate volume of whole blood previously collected during Step Two (~10 ml)

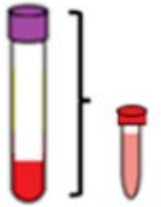
Step Eleven

- ❑ Gently invert the tube 10 times to mix saline and RBCs.
- ❑ Centrifuge the EDTA tube containing saline and RBCs at **2000 x g for 5 minutes** at 4° C

Step Twelve

- ❑ Using a clean transfer pipette, remove and discard the saline supernatant
- ❑ Repeat Steps 10-12 for a total of **two washes** (or until the supernatant is clear and not red).
- ❑ Ensure complete removal of the final saline supernatant. (*Note: It is better to remove a mm of RBCs with the saline rather than leaving any saline in the sample.)

Step Thirteen



- ❑ Label clear top cryovial with "WASHED RBC" label
- ❑ Aliquot 1.5 ml of washed RBCs to one labeled red capped cryovial
- ❑ Store washed RBC aliquot at -80 °C until shipment to NCRAD
- ***Note: spin, aliquot and freeze all samples within <u>2</u> hours of collection**

8.0 Cerebrospinal Fluid Collection and Processing

CSF samples should be collected in the morning before breakfast and after an overnight fast. There should be a minimum 6-hour fast before collection of biomarker fluids and CSF. Only water is permitted until blood draws and the lumbar puncture are completed.

There are general guidelines to follow regarding CSF Collection.

- Begin by confirming participant consented to lumbar puncture (LP) before scheduling the procedure and again prior to performing procedure.
- If LP and PET scan are done on the same day, LP should be completed prior to the PET scan; otherwise there should be at least 12 hours between LP and PET scan.
- LP should occur after, or a minimum of 72 hours prior, to an MRI scan.
- Do NOT use any extension tubing due to the tendency of manufactured plastic tubing to bind beta amyloid peptides and other important AD biomarkers.
- If LP was attempted but unsuccessful in obtaining CSF, a second attempt under fluoroscopy (if deemed appropriate by site clinician) will be permitted following approval from ADCS Clinical Operations.
- Site personnel should advise the participant that use of fluoroscopy (x-rays) involves exposure to radiation.
- Participants taking an anti-platelet agent (e.g. aspirin) may, at the discretion of the site clinician, be discontinued from that agent for a period of time prior to lumbar puncture and/or continue off agent for a period of time post LP. Participants who are taking anticoagulants (e.g. warfarin (Coumadin) and/or dabigatran (Pradaxa)) may not undergo an LP and are not suitable to participate in this study.
- Each study participant or a person designated to speak for them will be contacted by phone one day after the LP to confirm participant well-being and to query about any adverse events.
- Identify a physician (e.g., anesthesiologist) able to perform a blood patch for any participant who experiences a post lumbar puncture headache. Find out ahead of time who to call to schedule and perform a blood patch at your center, should the need arise. Ensure billing procedures are in place ahead of time.
- Ensure you have at least two “Lumbar Puncture Tray Kits” and sufficient “CSF Supplemental Supply Kit” provisions on hand prior to scheduling an LP visit. Also ensure adequate site-provided supplies (see above), including pelleted pelleted dry ice, are available. Check expiration dates on all supplies, especially lidocaine.

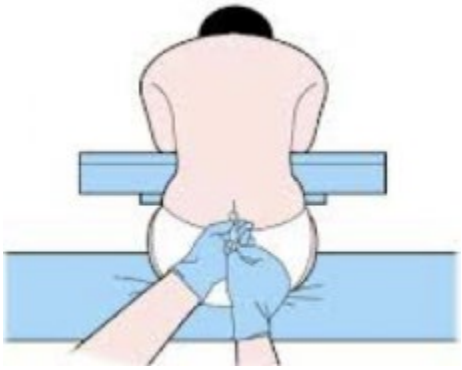
8.1 Scheduling the LP

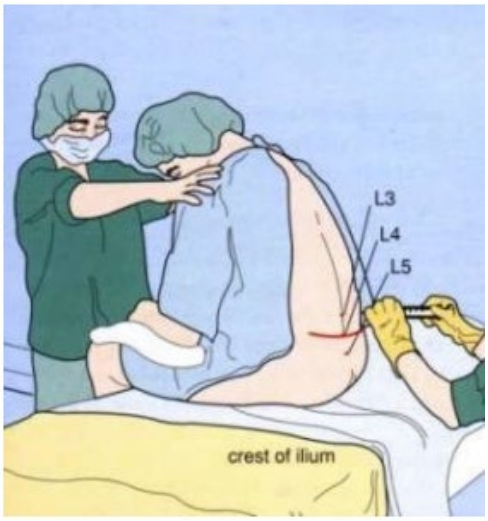
All LPs should be performed in the morning if possible. Availability of staff and facilities for next day blood patch should be considered when scheduling LPs. CSF amyloid levels can vary depending upon the time of day the sample is collected. It is important for the time of day of collection to remain consistent across study visits.

The LP should be rescheduled if the participant does not feel well or is febrile.

8.2 Performing the LP

The recommended position is sitting with curved back and head down. For comfort, a stool may be used to prop up the feet and legs. The same position should be used at follow-up LPs. It is critical to try to optimize positioning, and usually requires an assistant. Other positions and needles are allowed (e.g., when using fluoroscopy) but this should be recorded on the CSF Sample and Shipment Notification Form. A pillow may be placed under the head for comfort.

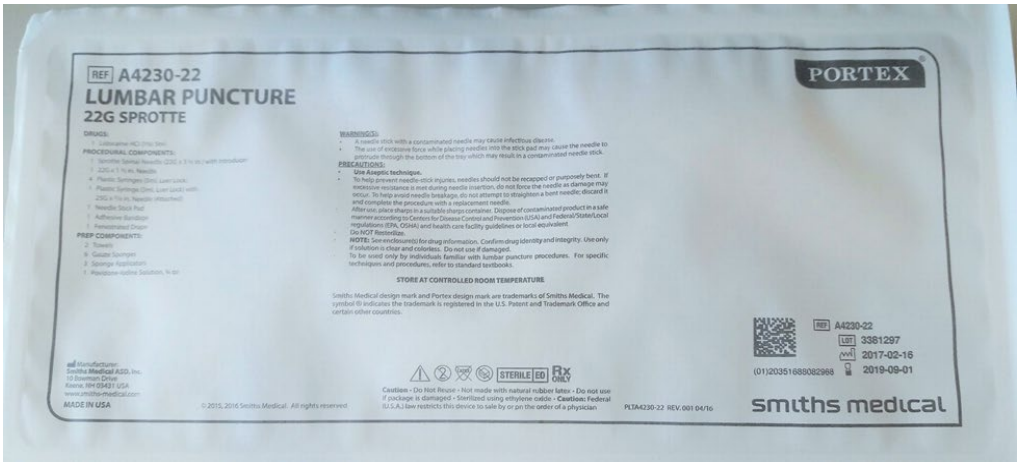




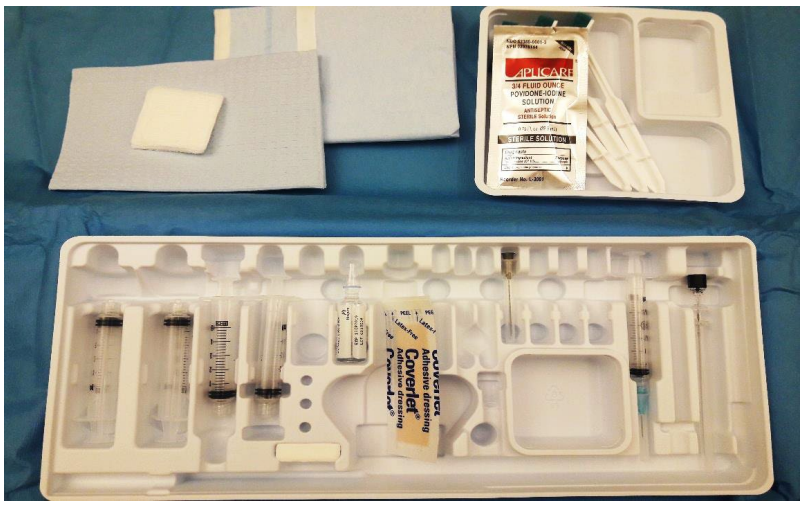
On the bedside table nearest where the person performing the lumbar puncture will sit, place a pair of sterile gloves (in their packaging) and a blue pad. Remove the contents of the lumbar puncture tray from the outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything wrapped until the person performing the lumbar puncture is seated.

Feel the outside of the lumbar puncture kit (still wrapped up) to determine which end contains the spongy swabs. Turn this end toward the person performing the lumbar puncture and begin un-wrapping the kit.

Lumbar Puncture Tray Kit Images



Exterior of LP Tray provided by NCRAD containing the 22 gauge Sprotte Needle with Introducer



Interior of LP Tray Provided by NCRAD

TOUCH ONLY THE OUTSIDE OF THE PAPER WRAPPER. When you grab an edge to unfold it, touch only the folded under portions of the outside of the wrapper. Also, don't let the outside of the wrapper touch any part of the inside.

- If you touch any part of the paper wrapper, or if any non-sterile object outside of the wrapper touches any part of the inside of the wrapper, throw the kit away and start over.
- If you are in any doubt as to whether the inside of the wrapper has been touched, throw the kit away and start over.

Cleaning the Lumbar Puncture Site

The lumbar puncture site is cleaned with Povidone-Iodine Topical Solution according to best standard medical practices.

Once the kit is successfully unwrapped, open the bottle of Povidone-Iodine Topical Solution somewhere away from the kit. Use an alcohol swab to remove any loose chunks of dried material off of the bottle top. You don't want anything to fall onto the open and sterile lumbar puncture kit. Pour enough Povidone-Iodine Topical Solution into the prep well to cover the bottom, about ¼ inch deep.

Maintaining the Sterile Field
An important aspect of assisting with a successful lumbar puncture is keeping the field sterile. If there are a number of staff members in the room, please be sure they do not accidentally contaminate the sterile field. Once the person performing the lumbar puncture has donned sterile gloves, additional help may be needed to obtain or un-wrap any new tubes, needles, or supplies.

Unwrapping the Sterile 15 and 50 ml Conical Tubes
Note that the 15 ml and 50 ml tubes into which CSF is collected and transferred come individually wrapped and are sterile inside and out. These wrappers should be peeled open by an assistant (not touching the tube) and the tube carefully dropped onto the LP tray or elsewhere in the sterile field in a manner that avoids contamination. Any additional needles or other individually-wrapped sterile items can be handled the same way.

- Do not drop any packaging onto the tray or sterile field.
- Do not let the item touch the outside of the packaging on its way to the tray.

Lidocaine, Syringe with Needle, Gauze Pads
Anesthesia is usually achieved within 2 minutes after injecting the lidocaine. Occasionally, the person performing the lumbar puncture will need to use more lidocaine to numb up a particular spot, or they may need to move to another spot entirely.

Hold the lidocaine bottle upside down and at a slight angle toward the person performing the lumbar puncture so that they can plunge the needle into the bottle and extract some lidocaine without touching you or the bottle. Use two hands to stabilize the bottle. If the person performing the LP requires additional sterile gauze, open the gauze pad the same way as the syringe and needle, by holding open the package so the person performing the lumbar puncture can grab the gauze without touching you or the package.

General CSF Collection Methods
LPs for CSF collection should be performed using a small caliber atraumatic needle. CSF should be obtained via gravity flow using the 22 gauge Sprotte needle, although aspiration through this or smaller needles is allowable. Prior approval from the Clinical Core is required before the aspiration method can be utilized. Sites must designate the method of CSF collection for data tracking purposes. It is recommended that CSF be obtained from participants in a sitting position. Alternate needles, positions or methods

(e.g., use of fluoroscopy) should be noted on the CSF Sample and Shipment Notification Form.

Collection of CSF by Gravity

After the spinal needle enters the L3-4 or adjacent intrathecal space and the stylet is withdrawn, CSF should flow freely. **Discard first 1-2 ml of CSF if blood tinged. If not blood tinged, collect first 1-2 ml of CSF into a 15 ml conical tube and pipette into the yellow cap cryovial for local lab. Collect 20 ml CSF total into the remaining two 15 ml conical tubes.**

Reminder: If the CSF is blood-tinged, the first 1-2 ml of CSF should be discarded (or more if needed) to clear the blood before collecting the 20 ml for CSF analysis. **15 ml is the required MINIMUM for CSF biomarker analysis.** If 15 ml is not obtained and provided to the NCRAD, document the reason for under-collection on the comments section of the CSF Sample and Shipment Notification Form.

Washcloths, Band-Aids, and Clean Up

After the person performing the lumbar puncture collects the last of the CSF, remove the needle and introducer and wash the Povidone-Iodine Topical Solution off the participant. A warm, wet washcloth can be used. A Band- Aid should be applied to the puncture site. The participant should lie flat for 30-60 minutes. Next, discard the LP kit following local guidelines, and dispose of sharp components in an appropriate sharps container.

Suggested management of post-lumbar puncture headache

Classic post-lumbar puncture (low pressure) headache typically begins 24-48 hours after dural puncture, and the headache is worse when the participant is upright (sits or stands) and improves when the participant is recumbent with the head **no higher** than the spinal cord.

Safety and comfort of the LP is maximized by the use of atraumatic needles. The protocol requires use of a 22 gauge Sprotte needle. Lumbar puncture is a standard procedure for collection of CSF but may be associated with pain during the performance of the procedure, comparable to the level of pain experienced during a blood draw. This is usually temporary and confined to the lower back. A persistent low-pressure headache may develop after lumbar puncture, probably due to leakage of CSF. If a post-LP headache persists it may need additional treatment, e.g. with fluids and analgesics. Uncommonly, a blood patch (injection of some of the participant's blood to patch the CSF leak) may be needed.

Prevention: Use of a small gauge and atraumatic needle with careful technique are helpful in preventing post-lumbar puncture headache. Having the participant refrain from exercise or strenuous activities (especially heavy lifting) and staying well-hydrated for 24 hours after the LP may minimize the chance of a lumbar puncture headache.

Treatment of headache after a lumbar puncture:

- Limit physical activity as much as possible for at least 24 hours post-procedure.
- Increase oral fluid intake. Caffeine may be helpful.
- Routine analgesics such as acetaminophen may be used.

Post-lumbar puncture headache often resolves with the above treatment. If the headache persists after 24 hours of this management, it will likely require a blood patch. A blood patch *typically* relieves the headache instantly.

8.3 Step by Step Summary of CSF Collection Procedure

1. Ensure all samples collected are appropriately labeled.

2. Print CSF Sample and Shipment Notification Form.

3. Confirm all supplies are available.

4. Label the thirteen clear-capped cryovials and one blue-capped cryovial with provided CSF Aliquot Labels. Do **NOT** open and label the 15 ml and 50 ml tubes that will be kept sterile to collect the CSF.

5. Pre-cool the centrifuge and pre-cool all fourteen labeled cryovials on wet ice. Do **NOT** pre-cool the 15 ml and 50 ml tubes that will be kept sterile to collect the CSF.

6. Measure vitals (participant lying down).

7. Record the time of LP and associated information on the CSF Sample and Shipment Notification Form.

8. Collect 20 ml CSF at the L3/L4 position (or adjacent position) using a 22 gauge Sprotte spinal needle via gravity flow with participant in upright position (or document alternate method on CSF Sample and Shipment Notification Form) following these steps:
    a. Collect initial 1-2 ml (if bloody, collect CSF until cleared of blood) using the 15 ml conical tube. If not bloody, transfer first 1-2 ml into yellow-capped cryovial for local lab.
    b. Collect an additional 20 ml CSF into the unlabeled and sterile 15 ml polypropylene tubes from the “CSF Supply Kit”. 15 ml is the required minimum.
    c. If using aspiration, use **ONLY** the polypropylene syringes included in the “Lumbar Puncture Collection Kit” and transfer directly into the unlabeled and sterile 15 ml polypropylene tube from the “CSF Supply Kit”. There are four 6 ml Luer lock polypropylene syringes in the “Lumbar Puncture Collection Kit.” Note this on the CSF Sample and Shipment Notification Form.

9. As one person takes the immediate post procedure vital signs, a second person should process the CSF as follows:

a. Place samples upright on wet ice and ensure samples are kept on wet ice for the entire time prior to processing. Preferably within 15 minutes of collection, centrifuge briefly at low speed (2000 x g, 10 min, 4°C) to pellet any cellular debris.
b. Using a clean transfer pipette, transfer CSF from both 15 ml conical tubes into a 50 ml conical tube, leaving the debris at the bottom of each 15 ml centrifuged tube. Gently invert the 50 ml conical tube 3-4 times to mix the sample.
c. Aliquot 1.5 ml volumes into the clear-capped cryovials. If a residual aliquot is created, aliquot into blue-capped cryovial. Document specimen number and volume on CSF Sample Notification Form.
d. Within 1 hour of CSF collection, samples need to be spun, aliquoted and in the freezer. Store CSF aliquots at -80°C until shipment. Record time of freezing on CSF Sample and Shipment Notification Form.

10. Provide food and drink to participant (participant may lay flat to minimize the chance of a post-LP headache).

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 and Shipment Notification Form (Appendix D).

CSF Aliquots (up to 12 possible)

CSF Preparation (20 ml total)

Step One

- Label cryovials with pre-printed specimen labels prior to collection.
- Pre-chill all cryovials on wet ice.

Step Two

- Collect initial 1-2 ml CSF for local lab testing.
- If bloody, collect CSF until cleared of blood).
- If not bloody, transfer into the yellow-capped cryovial. Send to local lab for cell count.

Step Three

- Collect 20 ml total (including the 1-2 ml for local lab testing) into two 15 ml conical tubes.

Step Four

- Place samples upright on wet ice until centrifugation begins.

Step Five

- Within 15 mins of collection, centrifuge samples at 4°C at 2000 x g for 10 minutes.

Step Six

- Using a clean transfer pipette, transfer all CSF into a new 50 ml conical tube leaving the pellet in the bottom.
- Gently invert the 50 ml conical tube 3-4 times to mix the sample.
- Aliquot 1.5 ml into 10 clear capped cryovials
- If residual aliquot is created, place sample into the blue capped cryovial and document the specimen number and volume on Sample Form.
- Store CSF aliquots upright at -80°C until shipment.
- Spin, aliquot, and freeze aliquots within 2 hours of collection.

9.0 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 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

9.1 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 pelleted dry ice.

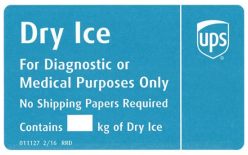
Specimens being shipped to NCRAD should be considered as Category B UN3373 specimens and as such must be tripled packaged and compliant with IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

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



9.1.1 NCRAD Packaging Instructions – Frozen Shipments

1. The Blood Sample and Shipment Notification Form (Appendix C) that is filled out during processing is required to be printed out and included in the physical shipping box.

2. A digital copy of The Blood Sample and Shipment Notification Form (Appendix C) is also required to be e-mail to alzstudy@iu.edu *prior* to shipping.
    a. **Important Note:** Neglecting steps 1 and 2 may result in delay of screening results and affect the quality of all specimens.

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

4. 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.

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

6. 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.

7. Fully cover the biohazard bags containing the cryovial boxes tubes with approximately 2 inches of pelleted dry ice.

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.**

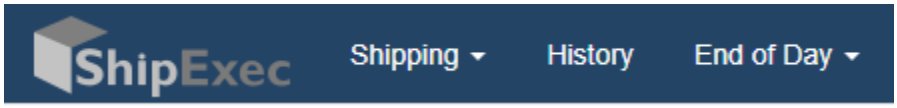
12. If possible, hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off. If storage in a -80°C freezer until UPS pick-up is not possible, package samples no more than 4 hours before the expected pick-up time.

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.

9.2 Frozen Shipping Instructions

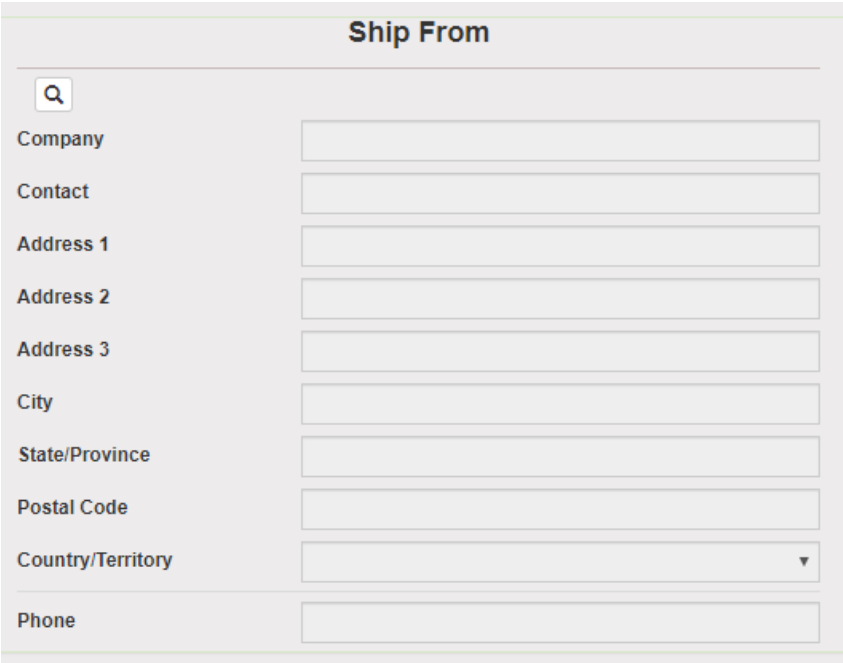
1. Log into the ShipExec Thin Client at kits.iu.edu/UPS.
    a. If a new user or contact needs access, please reach out to your study contact for access.

2. Click “Shipping” at the top of the page 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 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.

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

11.0 Appendices

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. Only send the GUID to NCRAD.

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).

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 at alzstudy@iu.edu

Appendix C: 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 #:** **1Z976R8W84**

Phone:_____ **Email:**_____

Study: BenfoTeam **Site ID:** _____ **Participant ID:** BENFO_____ **Sex:** ☐M ☐F **Year of Birth:** _____

GUID: _____ **Protocol Number**: ADC-061-BENFO

Visit: ☐**Screening** ☐**Baseline** ☐**Week 8** ☐**Week 72** ☐**Early Termination**

Dose: ☐**Pre-dose** ☐**Post-dose** **Time of Dose:**_____ (24-hour format)

KIT BARCODE

Blood Collection: (24-hour format)

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

Blood Processing: **Plasma & 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
Time spin started:	_____ [HHMM]	Duration of centrifuge:	_____ mins
Temp of centrifuge:	_____ °C	Rate of centrifuge:	_____ x g
Time aliquoted:	_____ [HHMM]	Number of **1.0 mL** plasma aliquots (purple cap): _____	Number **0.5 mL** plasma aliquots (purple cap) _____
Volume of residual plasma aliquot (less than 0.5 mL in blue cap):	_____ mL ☐N/A	Specimen number of residual plasma aliquot **(Last four digits)**:	_____ ☐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
Complete following fields for Baseline, Week 8, and Week 72 Visits Only:		Number of 1.0 mL whole blood aliquots created (yellow cap):	_____
Number of 1.5 mL washed RBC aliquots created (red cap):	_____	Duration of centrifuge:	_____ mins
Temp of centrifuge:	_____ °C	Rate of centrifuge:	_____ x g
Time aliquoted:	_____ [HHMM]	Time spin started	_____ [HHMM]

Notes:_____

Appendix D: CSF 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 #:**1Z976R8W84

Phone:_____ **Email:**_____

Study: BenfoTeam **Site ID:** _____ **Participant ID:** BENFO _____ **Sex:** ☐M ☐F **Year of Birth:** _____

GUID: _____ **Protocol Number**: ADC-061-BENFO

Visit: ☐**Baseline** ☐**Week 72**

KIT BARCODE

CSF Collection:

Date of Draw: _____ [MMDDYY]	Time of Draw: _____ [HHMM]
Date participant last ate: _____ [MMDDYY]	Time participant last ate: _____ [HHMM]
Collection process: ☐ Gravitational Specify if other method used:	Needle used to collect CSF: ☐ 22g Sprotte ☐ Other *(please specify)*: _____

CSF Processing:

Time spin started:	_____ [HHMM]
Duration of centrifuge:	_____ mins
Temp of centrifuge:	_____ °C
Rate of centrifuge:	_____ x g
Total amount of CSF collected (mL):	_____ mL
Time aliquoted:	_____ [HHMM]
# of 1.5 mL CSF aliquots created: **(Clear-capped cryovial)**	_____
If applicable, volume of CSF residual aliquot (less than 1.5 mL): **(Blue-capped cryovial)**	_____ mL
If applicable, specimen number of residual aliquot: **(Last four digits)**	_____
Time aliquots frozen:	_____ [HHMM]
Storage temperature of freezer:	_____ °C

Notes: _____