Nicotinic Acid for the Treatment of Alzheimer's Disease: A Phase 1b/2a Study

in collaboration with the

National Centralized Repository for Alzheimer's Disease and Related Dementias

Biospecimen Collection, Processing, and Shipment Manual of Procedures

Version 10.23.2024

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1.0 Abbreviations

AD	Alzheimer's Disease
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PHI	Protected Health Information
RBC	Red Blood Cells
CSF	Cerebrospinal fluid
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

2.0 Purpose

The collection of biofluids is an important part of the Nicotinic Acid for the Treatment of Alzheimer's Disease: A Phase 1b/2a 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 Nicotinic Acid study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

The following samples will be sent to NCRAD:

- Plasma
- Buffy Coat (DNA Extraction)
- Cerebrospinal fluid

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

These procedures are relevant to all study personnel responsible for processing specimens provided to NCRAD for the Nicotinic Acid 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

Erin Delaney, BS, CCRP, Clinical Research Coordinator
Phone: 317-278-1221
Email: eridelan@iu.edu

General NCRAD Contact Information
Phone: 1-800-526-2839/317-278-8413
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: www.ncrad.org
Study Webpage: www.ncrad.org/coordinate-studies/nicotinic-acid

Sample Shipment Mailing Address
NICOTINIC ACID 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 8.0 of this protocol.

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

3.3 NCRAD Holiday Observations

Date	Holiday
January 1	New Year's Day
3rd Monday in January	Martin Luther King, Jr Day
4th Monday in May	Memorial Day
June 19	Juneteenth
July 4	Independence Day (observed)
1st Monday in September	Labor Day
4th Thursday in November	Thanksgiving
4th 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 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 samples 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 Nicotinic Acid Laboratory Collection

4.1 Site Required Equipment

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

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

- 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)

4.2 Biospecimens Sent to NCRAD

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

4.2.1 Biofluid Collection Schedule

Biospecimen Collection Table

Biospecimen	Day 0, Day 60
Plasma	X
Buffy Coat (DNA)	X
CSF	X

Whole blood is collected in two collection tubes (two 10 ml purple-top EDTA tubes) for shipment to NCRAD. The 10 ml EDTA tubes are processed locally into plasma, and buffy coat fractions; they are then aliquoted, frozen at the study site, and shipped 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 on the NCRAD website at: https://ncrad.org/bank-samples/sample-management/recommended-consent-language copy of the consent form for each participant should be kept on file by the site investigator.

Collection Tube	Drawn At	Specimen Type	Aliquot Volume	Total Number of Aliquots	Cap Color
2 x EDTA (Purple-Top) Blood Collection Tubes (10 ml)	Day 0, Day 60	Plasma	1.5 ml plasma aliquots	Up to 7	Purple
		Residual Plasma	<1.5 plasma aliquot	1	Blue
		Buffy Coat	~1.0 ml buffy coat aliquots	2	Clear
1 x CSF (Blue-Top) Conical Tube (20 ml)	Day 0, Day 60	Local Lab CSF	1.0-2.0 ml	1	Yellow
		Processed CSF	1.5 ml	11	Clear
		Residual CSF	<1.5 ml	1	Blue

5.0 Specimen Collection Kits, Shipping Kits, and Supplies

NCRAD will provide: 1) Blood and CSF sample collection kits for research specimens to be stored at NCRAD, the Supplemental Supply Kit, the Frozen Shipment Kit and 2) clinical lab supplies (with the exception of pelleted dry ice and equipment supplies listed in Section 4.1). The provided materials include blood tubes, pipettes, boxes for plasma/buffy coat aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit number labels, Participant ID labels, and cryovial labels will all be provided by NCRAD. Details regarding the blood and CSF kits are found in this Manual of Procedures. Cryovial 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 6.1.

5.1 NCRAD Specimen Collection Kit Contents

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

Nicotinic Acid Collection Blood Kit

Quantity	Blood Kit Components
2	EDTA tube, 10ml with purple cap
6	Cryovial (2.0 ml) with purple cap
1	Cryovial (2.0 ml) with blue cap
2	Cryovial (2.0 ml) with clear cap
1	Centrifuge tube, 15ml
10	Preprinted Cryovial labels
5	Kit Number Labels
3	Participant ID Labels
1	2x4 Label
1	Cryovial box (holds up to 25 cryovials)
2	Disposable graduated transfer pipette
1	Resealable plastic bag

Nicotinic Acid CSF Collection Kits

Quantity	CSF Kit Components
11	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	50 ml Conical tube, individually wrapped
1	Disposable pipet (3.0 ml)
13	Preprinted Cryovial Label
1	Participant ID Label
1	Kit Number Label
1	2x4 Label
1	Resealable bag
1	Cardboard cryobox, 25 slot
1	LP tray, US, 22-gauge Sprotte

NCRAD Frozen Shipping Supply Kit

Quantity	Frozen Shipping Kit Components for Blood-Based Biomarkers
8	Plastic Biohazard bag with absorbent sheet (small)
1	UPS return airbill and pouch
1	Shipping box/Styrofoam container
1	UPS Blue Dry Ice Sticker
1	Fragile Label
1	UN3373 sticker
1	Resealable bag
1	2x4 label

Nicotinic Acid Supplemental Supply Kit

Quantity	Supplemental Supply Kit Components
10	EDTA tube, 10ml
50	Cryovial (2.0 ml) with purple cap
5	Cryovial (2.0 ml) with blue cap
50	Cryovial (2.0 ml) with clear cap
5	15 ml Centrifuge tube
10	50 ml Conical tube, Individually Wrapped
1	Sprotte 22Gx3.5” needle with introducer
10	Participant ID labels
5	Cryovial box (holds up to 25 cryovials)
5	Disposable graduated transfer pipette
3	Resealable plastic bag
5	Plastic Biohazard bag with absorbent sheet (small)
5	UN3373 stickers
5	Fragile Labels
5	UPS Blue Dry Ice Stickers
1	2x4 Label

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module
By Request	Cryovial box (holds up to 25 cryovials)
By Request	Cryovial (2.0 ml) with purple cap
By Request	Cryovial (2.0 ml) with blue cap
By Request	Cryovial (2.0 ml) with clear cap
By Request	UPS return airbill
By Request	Shipping container for dry ice shipment (shipping and Styrofoam box)
By Request	Plastic biohazard bag with absorbent sheet (small)
By Request	Disposable graduated transfer pipette
By Request	EDTA (Purple-Top) Blood Collection Tube (10 ml)
By Request	Centrifuge tube, 15ml
By Request	Warning label packet
By Request	UN3373 label
By Request	Dry ice shipping label
By Request	Fine Point Permanent Markers
By Request	Participant ID Labels

5.2 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/NicotinicAcid 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 available.

5.3 Kit Supply Pick Up

Kits supplies will be picked up at our TK Location
Address: 351 W 10th Street Room #316
MONDAY-FRIDAY BETWEEN 8:00AM – 5:00PM

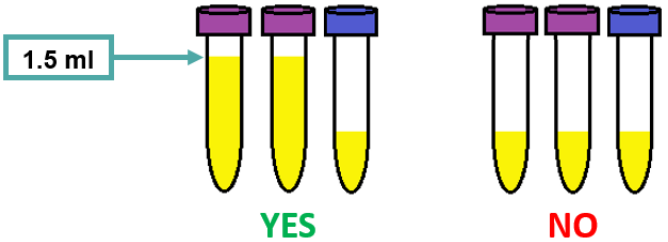
1. Enter through the main doors closest to the canal.
2. Turn left at the first hallway and there will be signs for elevators.
3. Take the elevator to the 3rd floor.
4. Pick up ordered kit supplies outside of room #316.

***Important Note**: Verify expiration dates for collection tubes prior to blood draw.*

5.4 Filling Cryovials

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

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



Please note: It is critical for the integrity of future studies using these samples that study staff note if an aliquot tube contains a residual volume (anything

under 1.5 ml). Please highlight that the aliquot contains a small volume by utilizing the blue cryovial cap provided in each kit. Please record the last four digits of the residual aliquot on the Biological Sample and Notification Form. If there are any unused cryovials, please do not send the empty cryovials to NCRAD. These unused cryovials (ensure labels are removed) can be saved as part of a supplemental supply at your site or the cryovials can be disposed of per your site's requirements.

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

Cap Color	Sample Type
Purple	Plasma
Clear	Buffy Coat
Blue	Residual sample plasma
Yellow	Local Lab CSF
Clear	Processed CSF
Blue	Residual CSF

6.0 Blood Collection and Processing Procedures

6.1 Labeling Samples

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

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

****Label Type Summary****

1. **Kit Number Label**
2. **Participant ID Label**
3. **Cryovial Label**

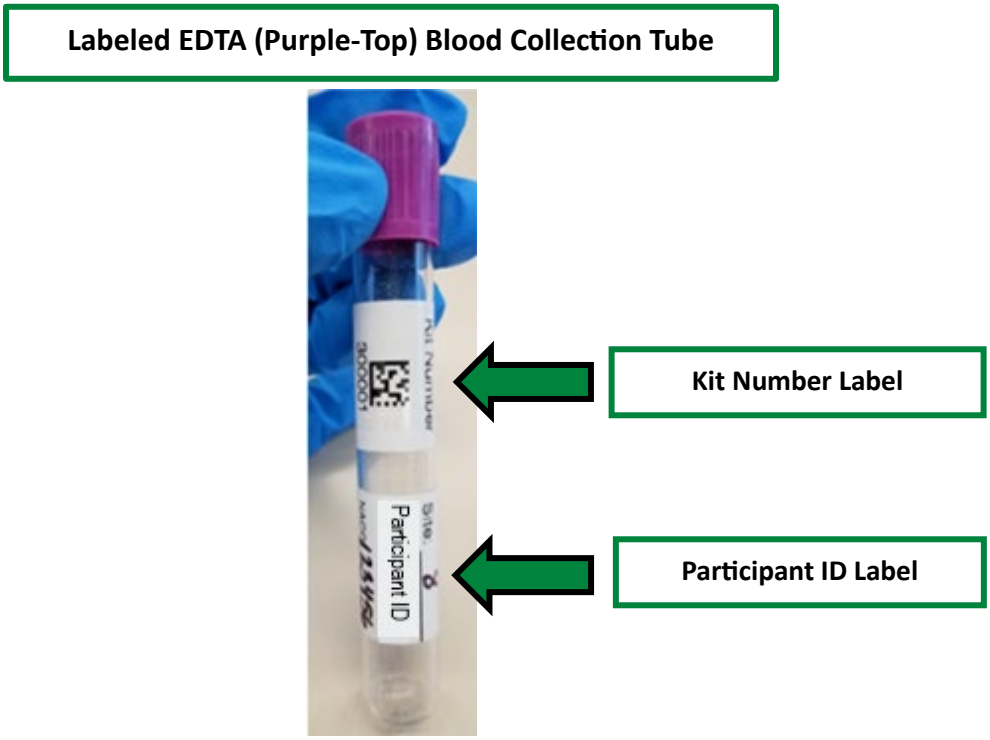
Kit Number Labels tie together all specimens collected from one participant at one visit. They should be placed on each cryobox, the EDTA collection tubes, and in the designated location on the Blood Sample and Shipment Notification Forms.

Participant ID Labels are used to document the individual's unique study participant ID and the blood processing staff initials. Place one label on each blood collection tube.

Place one **Cryovial Label** on each cryovial.

Important Note

Each collection tube will contain two labels: the kit number label and the 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 Participant ID label.

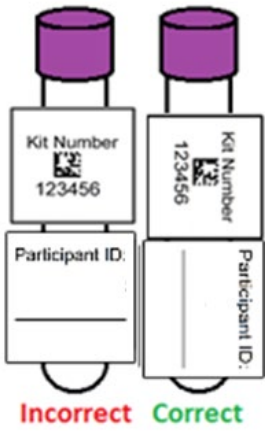


In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

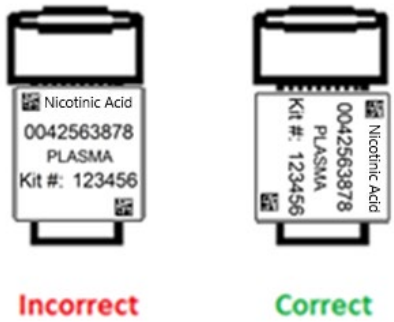
- Place all labels on **ALL** collection tubes and cryovials **BEFORE** sample collection. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Using a fine point permanent marker, fill-in and place the Participant ID labels on the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

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

Collection Tube Labeling Diagram



Cryovial Labeling Diagram



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

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

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

3. Place completed Participant ID Label and preprinted **Kit Number** label on each purple-top EDTA tube. Place preprinted **PLASMA** cryovial labels on the six 2 ml cryovial tubes with purple caps and one 2 ml cryovial tube with blue cap (if necessary, for residual). Place preprinted **BUFFY COAT** cryovial label on the 2 ml cryovials with clear caps.

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 last collection tube.

   d. Make sure tube additives found on the inside surface of the EDTA tube are not in contact with the 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.

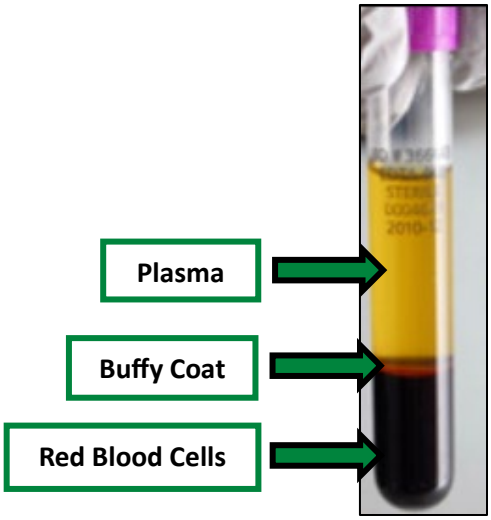
6. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8-10 times.

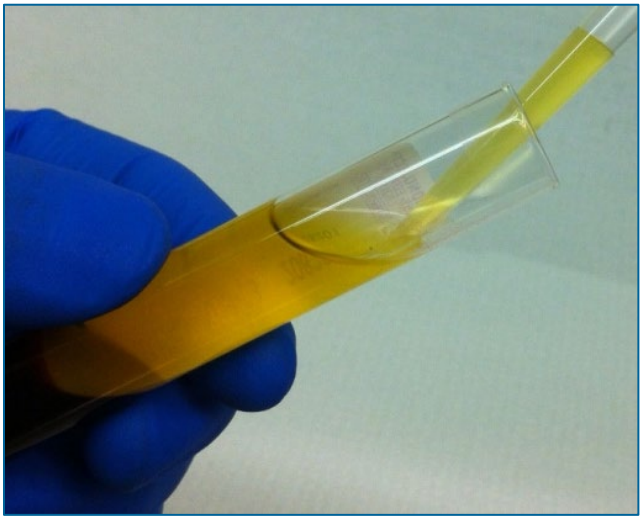
7. Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.

8. 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 A to calculate RPM.)**
   a. Equivalent rpm for spin at 2000 x g
   b. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
   c. Record original volume drawn for each tube in spaces provided on the Biological 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.
   e. Record time aliquoted on the Biological Sample Shipment and Notification Form.

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 red blood cells at the bottom of the collection tube.

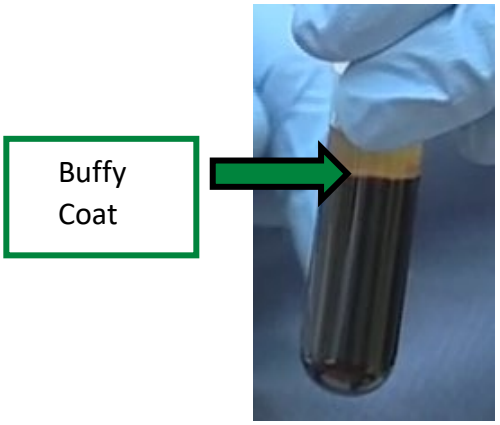
10. Each EDTA tube should yield, on average, 4-5 ml of plasma. Transfer plasma from both EDTA tubes into the 15 ml conical tube and gently invert 3 times. Aliquot 1.5 ml plasma per cryovial. Be sure to only place **plasma** in cryovials with purple caps and labeled with **PLASMA** labels**.** Place residual plasma (<1.5 ml) in the blue-capped cryovial. **If a residual aliquot (<1.5 ml) is created, document the specimen number and volume on the Biological Sample and Shipment Notification Form.**

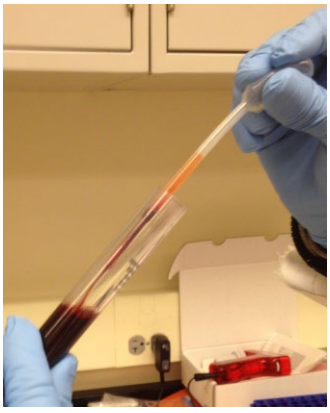




NOTE: When pipetting plasma from the EDTA tube into the 15 ml conical tube, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

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 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 EDTA tube, placing this buffy coat into the second clear-capped cryovial.





Buffy Coat Aliquot
(Clear-capped cryovial)

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

14. Record the specimen number 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 7 possible) and Buffy Coats (2)

Plasma and Buffy Coat Preparation EDTA Purple-Top Tube (10 ml)

Step One

- Store tubes at room temp.
- Each tube should be labeled with Collection Tube and Site and Participant Labels.

Step Two

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

Step Three

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

Step Four

- Place thoroughly mixed tube on wet ice until centrifugation begins.

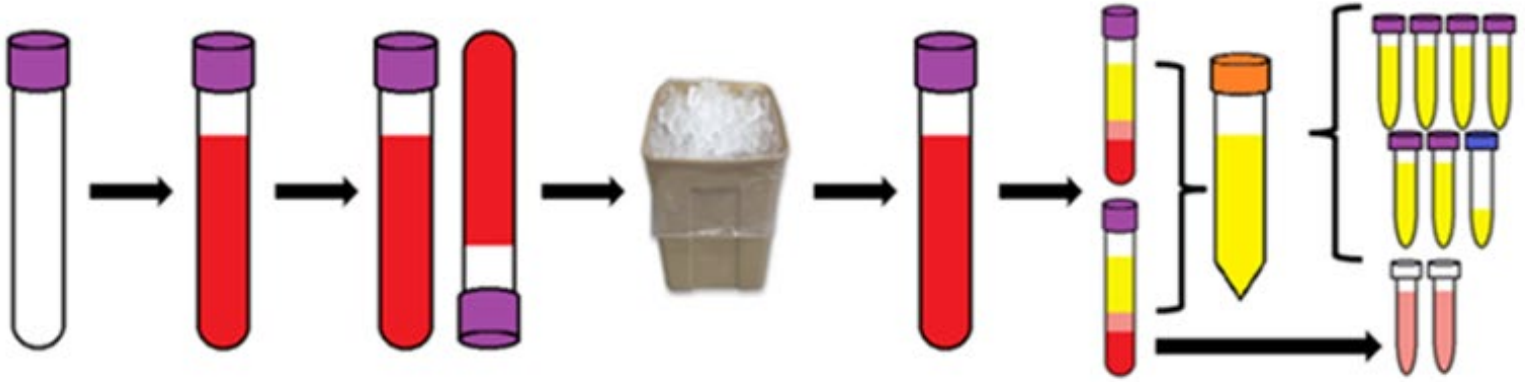
Step Five

- Centrifuge samples at 2000 x g for 10 minutes at 4°C.

Step Six

- Pool all plasma from the 2 EDTA tubes into a 15 ml conical tube and invert gently 3 times to mix the plasma.

Step Seven



- Label purple-capped cryovials with "PLASMA" labels. Aliquot 1.5 ml plasma into each cryovial.
- If residual aliquot is created, document specimen number and volume on Sample Form.
- Store plasma aliquots upright at -80°C until shipment to NCRAD.

Step Eight

- Label clear-capped cryovials with "BUFFY COAT" labels.
- Using a clean transfer pipette, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat from each EDTA tube into its own cryovial.
- Store buffy coat aliquots upright at -80°C until shipment to NCRAD.
- Spin, aliquot, and freeze all plasma and buffy coat aliquots within 2 hours of collection.

7.0 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 Principal Investigator.
- 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 dry ice, are available. Check expiration dates on all supplies, especially lidocaine.

7.1 Scheduling the LP

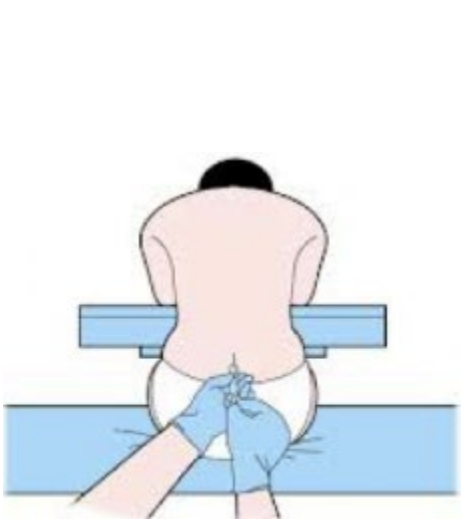
All LPs should be performed in the morning if possible. Availability of staff and facilities for next day blood patch should be considered when scheduling LPs. CSF

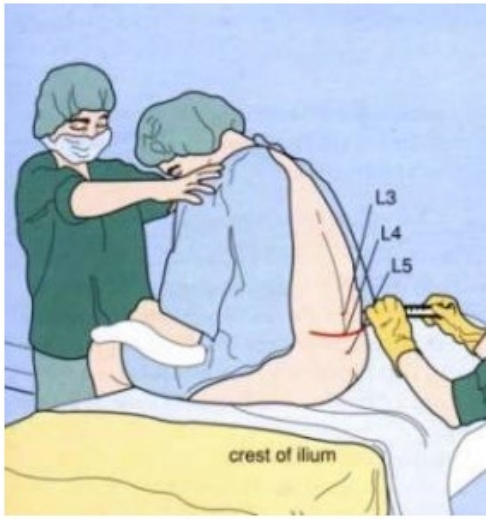
amyloid levels can vary depending upon the time of day the sample is collected. It is important for the time of day of collection to remain consistent across study visits.

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

7.2 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.

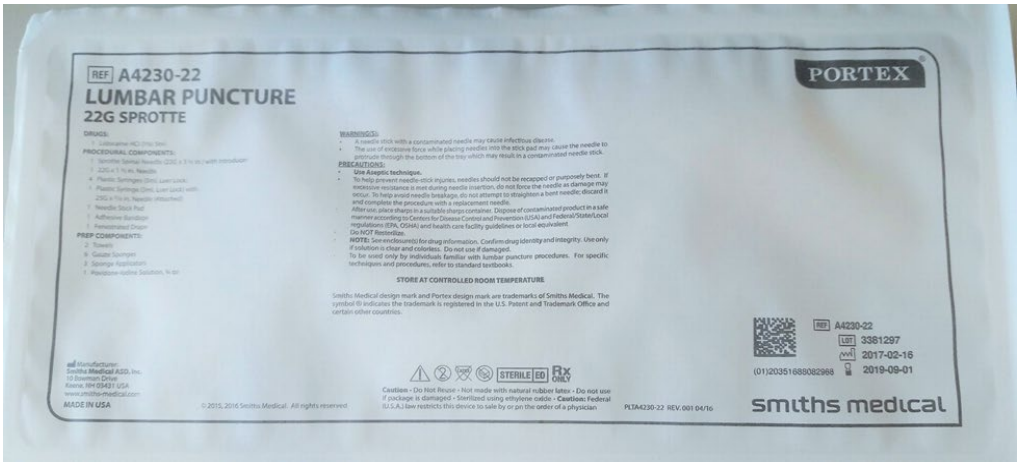




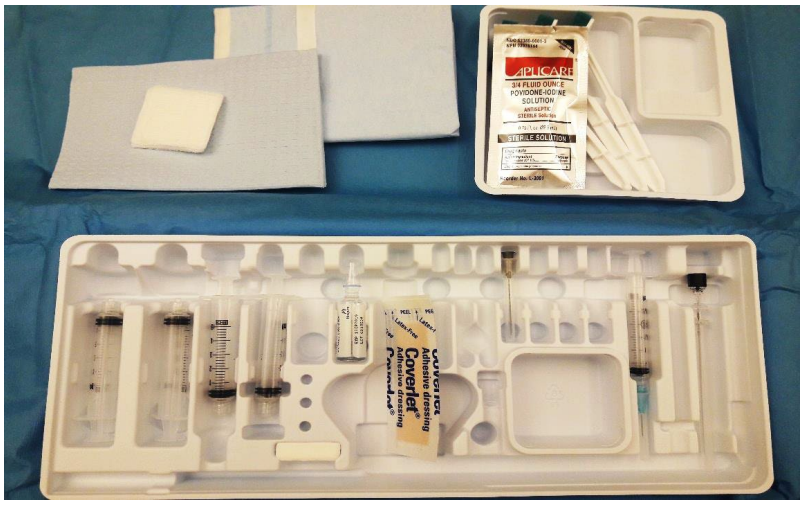
On the bedside table nearest where the person performing the lumbar puncture will sit, place a pair of sterile gloves (in their packaging) and a blue pad. Remove the contents of the lumbar puncture tray from the outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything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

7.3 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 C).

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 11 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.

8.0 Packaging & Shipping Instructions

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

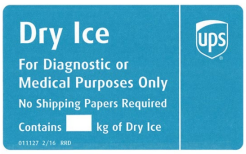
In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by NCRAD for each sample type. Investigators and clinical coordinators for each project are responsible to ensure the requested amounts of each fluid are collected to the best of their ability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

8.1 Frozen Packaging Instructions

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

***** 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



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

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

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

8.1.1 NCRAD Packaging Instructions – Frozen Shipments

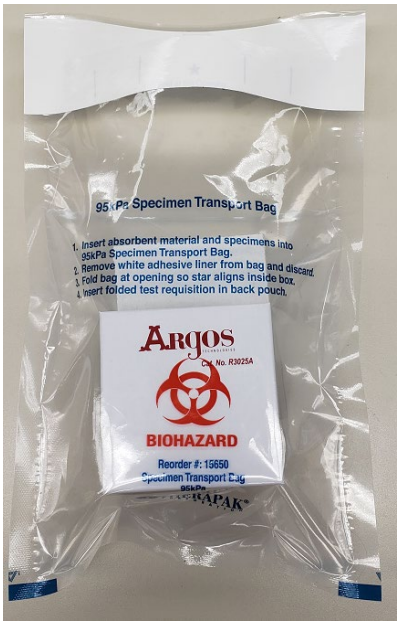
1. If possible, hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off. If storage in a -80°C freezer until UPS pick-up is not possible, package samples no more than 4 hours before the expected pick-up time.

2. Contact UPS to confirm service is available and schedule package to be picked up.

3. Notify NCRAD of shipment by emailing NCRAD coordinators at alzstudy@iu.edu. Attach the following to the email:
    a. Completed Sample Form (Appendix B) to the email notification (email NCRAD coordinator prior to shipment to receive sample form).
    b. If email is unavailable, please call NCRAD at 1-800-526-2839/317-278-8413 and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.

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

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



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

7. Place the biohazard bags into the provided Styrofoam-lined shipping container on top of the pelleted dry ice. Please ensure that cryovial boxes are placed so the cryovials are upright in the shipping container.
    a. If needed, cryovial boxes can be placed into the shipper in layers with pelleted dry ice between each layer of samples to fit 8 cryovial boxes in each shipper.

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

9. After the samples have been placed into the shipping container, fill the inner Styrofoam with plenty of dry ice pellets to ensure the frozen state of the specimens during transit.

10. Replace the lid on the Styrofoam carton. Place a copy of the completed Blood Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each participant specimen,

then close and seal the outer cardboard shipping carton with packing tape.

11. 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.

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

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

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

13. Hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off.

14. 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.

NICOTINIC ACID at NCRAD
Indiana University School of Medicine
351 W. 10th St. TK-342
Indianapolis, IN 46202

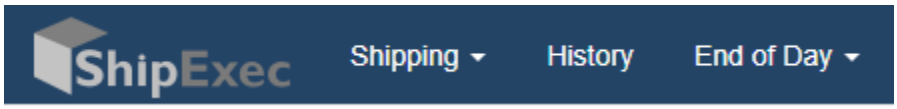
15. Use UPS tracking to ensure the delivery occurs as scheduled and is received by NCRAD.

8.2 Frozen Shipping Instructions

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.

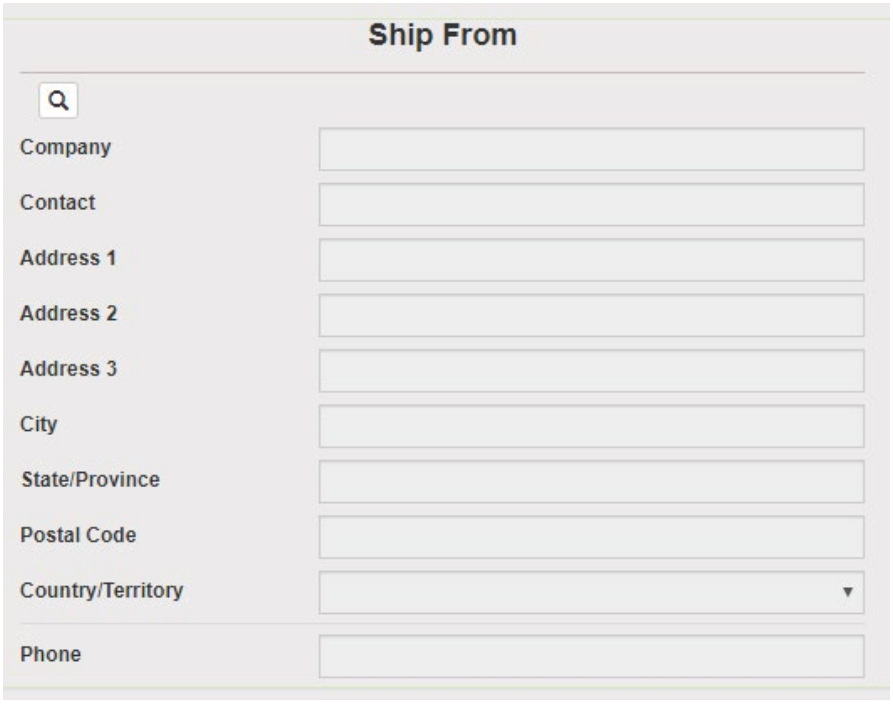
2. Log into the ShipExec Thin Client at kits.iu.edu/UPS.
    a. If a new user or contact needs access, please reach out to your study contact for access.

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



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

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



    a. Search by Company (site), Contact (name), or Address 1 (first line of your site’s street address). Click Search.
    b. Click Select to the left of the correct contact information.

6. Verify that both the shipping information AND study reference are correct for this shipment.
    a. If wrong study contact or study reference, click Reset in the bottom right of the screen to research for the correct information.

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

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

9. Print the airbill that is automatically downloaded.
    a. To reprint airbill, click History at the top left of the page.
        i. Shipments created from the user that day will automatically populate. If shipments from a previous day need to be located, search by ship date.
        ii. Locate the correct shipment, and click on the printer icon to the left of the tracking number under “Action” to reprint the airbill
        iii. Click print icon on right side of the tracking number line.

10. Fold airbill, and place inside plastic UPS sleeve. Peel the back off of the UPS sleeve, and stick the sleeve to the package.

9.0 Data Queries and Reconciliation

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

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

10.0 Appendices

Appendix A: Rate of Centrifuge Worksheet

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

Submitter Information

Name: **Site:**

Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

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

Calculating RPM from G-Force:

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

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

Comments:

Please send this form to NCRAD Study Coordinator

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

Appendix B: Blood Sample and Shipment Notification Form

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

To: Kelley Faber Email: alzstudy@iu.edu Phone: 1-800-526-2839/317-278-8413

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

Phone:_____ **Email:**_____

Study: Nicotinic Acid **Participant ID**: _____

Year of Birth: _____ **Sex:** ☐ M ☐ F

Visit: ☐ **Day 0** ☐ **Day 60**

KIT BARCODE

Blood Collection:

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

Blood Processing:

Plasma & Buffy Coat (EDTA Tube)

Original blood volume of EDTA #1:	_____	Original blood volume of EDTA #2:	_____ mL
Time spin started:	_____ [HHMM]	Duration of centrifuge:	_____ mins
Temp of centrifuge:	_____ °C	Rate of centrifuge:	_____ x g
Time aliquoted:	_____ [HHMM]	Number of 1.5 mL plasma aliquots created (purple cap, up to 6):	_____
If applicable, volume of residual plasma aliquot (less than 1.5 mL in blue cap):	_____ mL ☐ N/A	If applicable, 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):**	_____	Buffy coat #2 volume:	_____ mL
Time aliquots placed in freezer:	_____ [HHMM]	Storage temperature of freezer:	_____ °C

Notes:_____

Appendix C: 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: Nicotinic Acid **Participant ID:**_____

Year of Birth: _____ **Sex:** ☐ M ☐ F

Visit: ☐ **Day 0** ☐ **Day 60**

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: _____