BioSTAC Protocol Update: V03.2021

Section	Change
3.2	Removed Usha Sirimalle as a NCRAD contact.
3.2	Updated NCRAD fax number.
8.2.1	Renamed “NCRAD Packaging Instructions—Frozen Shipments”. Removed shipping information
8.3	Frozen Shipping Instructions added to include new UPS steps
Appendix A	Updated NCRAD fax number.

Biomarker Standardization at Alzheimer's Centers (BioSTAC)

in collaboration with

University of Pennsylvania
and
National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD)

Biospecimen Collection, Processing, and Shipment Manual of Procedures

Version 03.2021

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1.0 ABBREVIATIONS

CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
LP	Lumbar Puncture
NACC	National Alzheimer's Coordinating Center
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
UPenn	University of Pennsylvania

2.0 PURPOSE

The purpose of this manual is to provide the Biomarker Standardization at Alzheimer's Centers (BioSTAC) staff (PIs, study coordinators, and the sample collection and processing teams) at the various study sites with instructions for collection and submission of biological samples for BioSTAC study visits. It includes instructions for biospecimen submission to the University of Pennsylvania (**UPenn**) and the National Centralized Repository for Alzheimer's Disease and Related Dementias (**NCRAD**) located at Indiana University. The following samples may be collected at each study visit:

- Plasma
- Buffy Coat (for DNA extraction)
- CSF

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 **UPenn** and **NCRAD**.

These procedures are relevant to all study personnel responsible for processing blood specimens to be submitted to **UPenn** and **NCRAD** for the BioSTAC protocol.

3.0 CONTACT INFORMATION

3.1 UPenn Contacts

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NCRAD Contact Information
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Fax: 317-321-2003
alzstudy@iu.edu
www.NCRAD.org

Sample Shipment Mailing Address
Magda Korecka
University of Pennsylvania
Biomarker Research Laboratory
3400 Spruce Street, 7 Maloney South
Philadelphia, PA 19104
Phone: 215-662-6266

Hours of Operation
8am-4:30pm Eastern Time
Monday-Friday

Ambient samples must be shipped to UPenn Monday-Thursday ONLY!

Sample Shipment Mailing Address
BioSTAC at NCRAD
IU School of Medicine
351 W. 10th St TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

Hours of Operation
8am-5pm Eastern Time
Monday-Friday

Frozen samples must be shipped to NCRAD Monday-Wednesday ONLY!

3.3 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
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 1st, both **UPenn** and **NCRAD** will be closed, re-opening for normal operations on January 2nd. If at all possible, biological specimens should **NOT** be collected and shipped to **UPenn** or **NCRAD** after the second week in December. Should it be necessary to ship samples **UPenn** or **NCRAD** during this period, please contact the corresponding staff before December 20th so that they can arrange to have staff available to process incoming samples.

- 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 BioSTAC 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
- Tourniquet
- Alcohol prep pad
- Gauze pad
- Bandage
- Butterfly needles and hub
- Microcentrifuge tube rack
- Sharps bin and lid
- Dry ice
- Wet ice
- Wet ice bucket

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 RCF (x g) with refrigeration to 4°C
- -80°C Freezer

In order to ship frozen specimens to NCRAD, you must provide:

- Dry ice (approximately 10 lbs per shipment)

4.2 Biospecimens Collected

Biospecimens collected include whole blood and CSF. Both biospecimens are collected following a minimum 6 hour fast. Please refer to the table below for the biospecimen schedule.

	Visit
Plasma	X
Buffy Coat (for DNA)	X
CSF	X

Whole blood will be collected into one 10 mL collection tube (purple-top EDTA tube) provided by NCRAD. The purple-top EDTA tube is processed locally into plasma and buffy coat fractions, aliquoted, frozen at the study site, and then shipped to NCRAD. Sites will typically send four aliquots of plasma, but can send additional plasma aliquots, if available.

CSF will be collected into one false bottom, low protein binding CSF collection tube and one or two cryovials provided to sites through NCRAD. One aliquot will be shipped ambient to **UPenn**. At least 1.0 ml of CSF will be frozen at the study site and then shipped to NCRAD. Any additional CSF that you collect may be maintained at your Center or 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. A copy of the consent form for each subject should be kept on file by the site investigator.

Guidelines for the processing, storage location, and timing of sample collection are listed in the following tables.

4.3 Biospecimen Collection Charts

4.3.1 Blood Collection

Collection Tube	Specimen Type	Aliquot Volume	Number of Samples Shipped	Shipping Destination	Shipping Temperature
EDTA (Purple-Top) Blood Collection Tube (10 ml)	PLASMA	1.0 ml plasma aliquots	4*	**NCRAD**	Frozen
	BUFFY COAT	1.0 ml buffy coat aliquot	1	**NCRAD**	Frozen

** sites can send **NCRAD** additional plasma aliquots, if available*

4.3.2 Cerebrospinal Fluid Collection

Collection Tube	Specimen Type	Aliquot Volume	Number of Samples Shipped	Shipping Destination	Shipping Temperature
False bottom, low protein binding CSF collection tube	CSF	2.0-2.5 ml CSF aliquot	1	**UPenn**	Ambient
2 ml cryovials or syringe	CSF	1.0 ml CSF aliquot *(minimum)*	1*	**NCRAD**	Frozen

** sites can send **NCRAD** additional CSF aliquots, if available*

If a sample is not obtained at a particular visit, this should be recorded in the Comments/Notes section of the **UPenn** and/or **NCRAD** web-based sample forms (paper copy of NCRAD sample form found in Appendix C).

5.0 Specimen Collection Kits, Shipping Kits and Supplies

Research specimen collection kits as well as clinical lab supplies (except dry ice and equipment supplies listed above) will be provided by **NCRAD**. These materials include blood tubes, lumbar puncture (LP) trays, boxes for plasma/buffy coat/CSF aliquot storage and shipment, as well as shipping labels to send materials to **NCRAD** and **UPenn**. Barcoded kit labels, site and NACC ID labels, and Specimen Labels will all be provided by **NCRAD**. Specimen Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 6.1 and Section 6.2.

5.1 Specimen Collection Kit Contents

Collection kits contain the following (for each subject) and provide the necessary supplies to collect samples from a given subject. 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.*

BioSTAC Collection Kit

Quantity	BioSTAC Collection Kit Components
1	EDTA (purple-top) blood collection tube (10 ml)
4	Cryovial (2 ml) with purple cap
2	Cryovial (2 ml) with gray cap
1	False bottom, low protein binding CSF collection tube
2	Disposable graduated transfer pipette
1	Pre-printed **UPenn** CSF Specimen Label
7	Pre-printed **NCRAD** Cryovial Labels
4	Pre-printed Kit Number Labels
2	Labels for handwritten Site and NACC ID
1	Cryovial box (holds up to 25 cryovials)

BioSTAC LP Kits*

**Sites must specify 22 or 24 gauge Sprotte needle kit when ordering from NCRAD. Sites using a needle other than Sprotte will need to provide their own needles and document which ones are used.*

Quantity	LP Kit Components
1	Sprotte needle, 22 or 24 gauge 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
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

UPenn Ambient Shipping Supply Kit

Quantity	Ambient Shipping Kit Components
1	Plastic biohazard bag with absorbent sheet
1	Small IATA shipping box with insulated cooler
1	Small refrigerant pack
1	Aqui-Pak 6 tube absorbent pouch
1	UN3373 Biological Substance Category B label
1	List of contents card
1	**UPenn** UPS return airbill
1	UPS Clinic Pak

NCRAD Frozen Shipping Supply Kit

Quantity	Frozen Shipping Kit Components
3	Plastic Biohazard bag with absorbent sheets
1	**NCRAD** UPS return airbill
1	Shipping box/Styrofoam container
1	Warning label packet

BioSTAC Supplemental Supply Kit

Quantity	BioSTAC Supplemental Kit Components
5	EDTA (purple-top) blood collection tube (10 ml)
5	Cryovial box (holds up to 25 cryovials)
5	False bottom, low protein binding CSF collection tube
20	Cryovial (2 ml) with purple cap
10	Cryovial (2 ml) with gray cap
10	Disposable graduated transfer pipette
5	UPenn UPS return airbill
5	NCRAD UPS return airbill
5	Warning label packet
5	Site and NACC ID label

We realize there may be instances where additional supplies are needed; therefore, one supplemental kit will be provided with the initial kit shipment. Replacement supplemental kits can be requested on the kit website. In addition, individual supplies can be requested on the kit website.

Individual Supplies

Quantities	Item
Per Request	EDTA (purple-top) blood collection tube (10 ml)
Per Request	Cryovial box (holds up to 25 cryovials)
Per Request	Cryovial (2 ml) with purple cap
Per Request	Cryovial (2 ml) with gray cap
Per Request	False bottom, low protein binding CSF collection tube
Per Request	Plastic Biohazard bag with absorbent sheet
Per Request	UPS return airbill
Per Request	Warning label packet
Per Request	Ambient shipping supplies (all come as one unit)
Per Request	Shipping container for frozen shipment (shipping and Styrofoam box)
Per Request	Site and NACC ID label

5.2 Kit Supply to Study Sites

Each individual site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out so you are prepared for study visits. Please go to: http://kits.iu.edu/BioSTAC/ to request additional kits and follow the prompts to request the desired supplies. Options include ordering specific number of kits (BioSTAC Blood Kit, BioSTAC CSF Kit, Frozen Shipping Supply Kit and/or a BioSTAC Supplemental Kit) or individual supplies. Please allow **TWO weeks** for kit orders to be processed and delivered.

6.0 Blood Collection and Processing Procedures

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

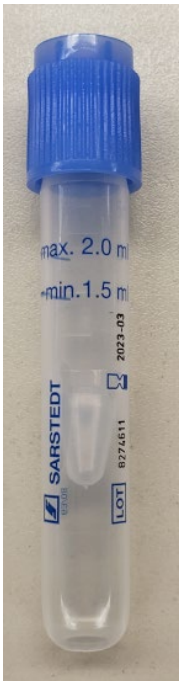
In order to ensure the highest quality samples are collected, processed, and stored, it is essential to follow the specific collection, processing, and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies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

6.1 Labeling UPenn CSF Sample

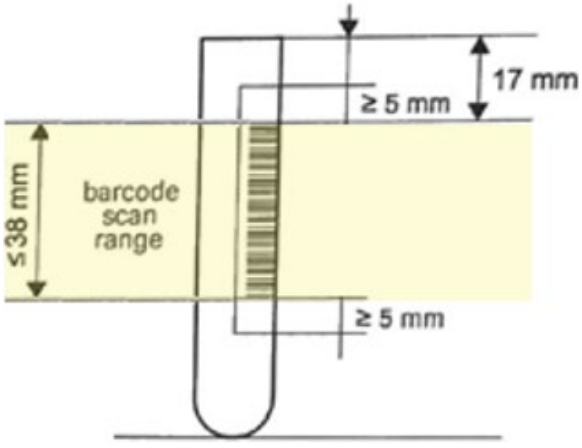
UPenn Specimen Label

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

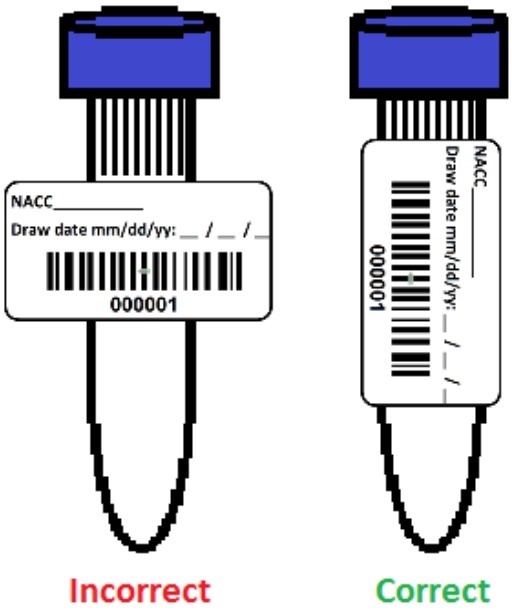
- Using a fine point permanent marker, fill-in and place the **UPenn** specimen label on the collection tube immediately following collection to keep the tube sterile.
- Place label approximately in the middle of the tube to allow for automated barcode scanning (barcode scan range highlighted in yellow in labeling diagram below).
- 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.



UPenn CSF Cryotube



Label Alignment for UPenn CSF Cryotube

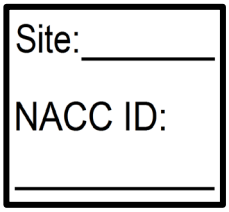


6.2 Labeling NCRAD Samples

****Label Type Summary****

1. **Kit Number Label**
2. **Site and NACC ID Label**
3. **Cryovial Label**

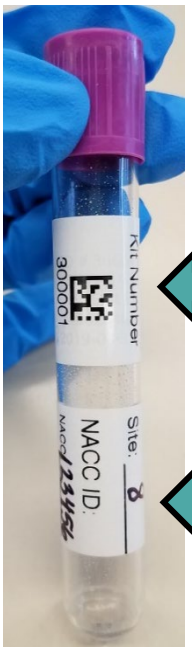
Kit Number Labels tie together all specimens collected from one subject at one visit. Place one kit number label on the EDTA collection tube before specimen collection. Also, place one kit number label on the subject's corresponding cryovial box.



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

BioSTAC
Plasma
Kit: 300001

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



Kit Number Label

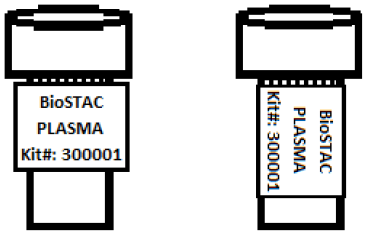
Site and NACC ID

EDTA Tube

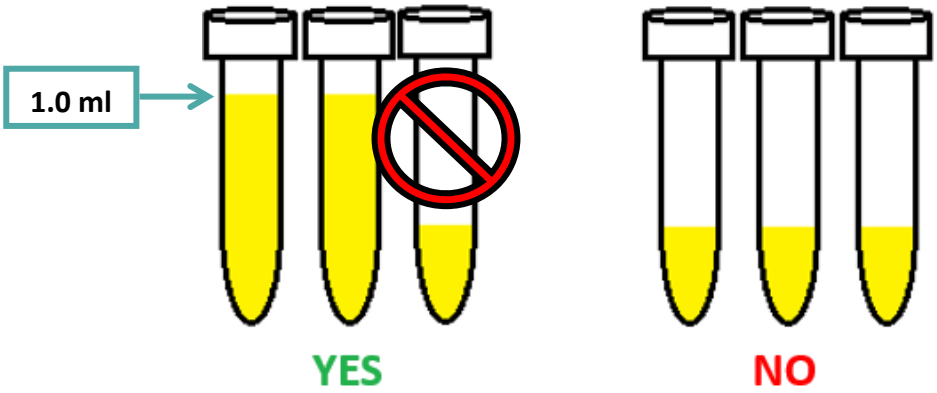
In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place cryovial labels on **ALL** cryovials **BEFORE** sample collection, processing, or freezing. 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 NACC ID label on the EDTA tube **BEFORE** sample collection or processing. This label is in addition to the kit number label. **DO NOT** place NACC ID labels on any cryovials.
- Place cryovial label **horizontally** on the tube (wrapped around sideways if the tube is upright) and just below the cap. **DO NOT** cover the barcode on the cryovial with the cryovial label (see following diagram).
- Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label.

6.3 Filling Aliquot Tubes (Plasma, Buffy Coat, 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 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.



Please note: It is critical for the integrity of future studies using these samples that study staff **not submit** residual aliquot tubes (any volume less than 1.0 ml) to NCRAD.

Please do not send any empty, unused cryovials to NCRAD. These unused cryovials can be disposed of per your site's requirements.

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

Blood should be collected the same day as CSF, following a minimum 6 hour fast.

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

2. Place completed Site and NACC ID Label and pre-printed kit label on the purple-top EDTA tube. Place pre-printed kit label on the Blood/CSF Sample Processing Form (Appendix C). Place pre-printed **PLASMA** cryovial labels on

the four 2 ml cryovials with purple caps. Place pre-printed **BUFFY COAT** cryovial label on the one 2 ml cryovial with a gray cap.

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

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

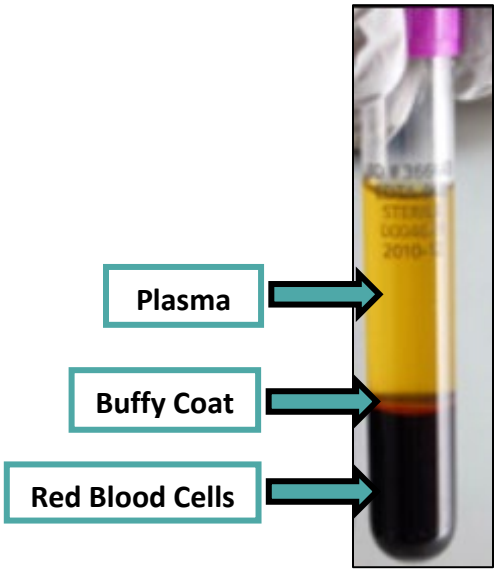
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
   a. If complications arise during the blood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

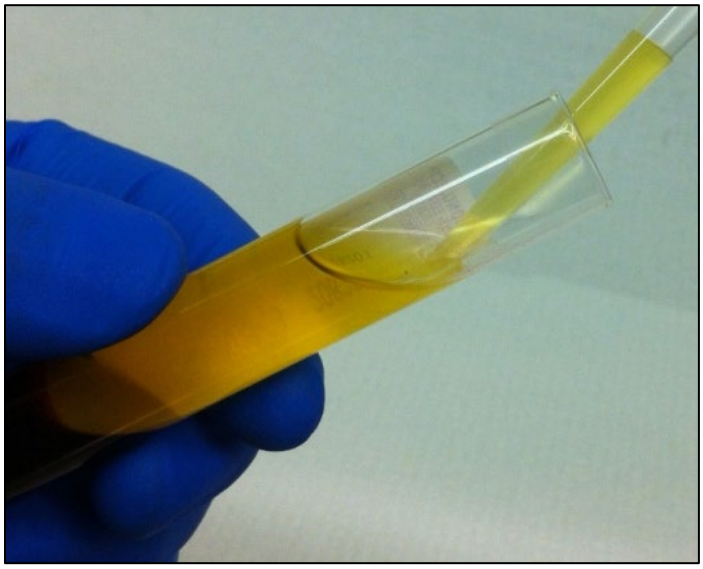
5. Immediately after blood collection, **gently invert/mix (180 degree turns) the EDTA tube 8-10 times**.

6. **Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.**
   a. 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).**
   b. While centrifuging, remember to record all times, temperatures and spin rates for documentation on the Blood/CSF Sample Processing Form (Appendix C).
   c. **Plasma samples need to be spun, aliquoted, and placed in the freezer within 1 hour from the time of collection.**
   d. Record time aliquoted on the Blood/CSF Sample Processing Form (Appendix C).

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

8. Transfer plasma into the pre-labeled cryovials with purple caps. The EDTA tube should yield, on average, 4-5 ml of plasma. Aliquot 1.0 ml into each purple-capped cryovial. Be sure to only place **plasma** in cryovials labeled with the **PLASMA** label and purple caps.





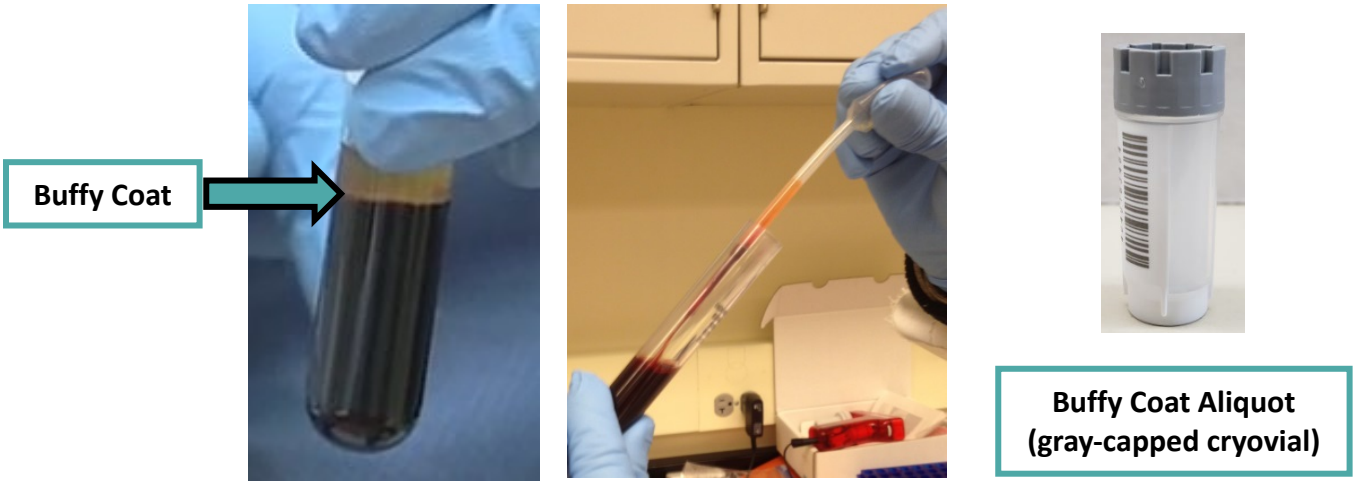
NOTE: When pipetting plasma from the EDTA tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

9. Place the labeled cryovials in the 25-slot cryovial box and place upright on dry ice.

Plasma Aliquots

10. After plasma has been aliquoted, pipet the buffy coat layer into the labeled, gray-capped cryovial using a clean pipette. All of the buffy coat should be

collected and placed into one cryovial. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to place buffy coat into the cryovial with the gray cap and **BUFFY COAT** label.



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

12. Place the buffy coat aliquot in the 25 cell cryovial box and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Blood/CSF Sample Processing Form (Appendix C).

Plasma and Buffy Coat Preparation
EDTA Purple-Top Tube (10 ml) for shipment to NCRAD

Step One

- Store tubes at room temp.
- Each tube should be labeled with NACC ID Label and Kit Number Label.

Step Two

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

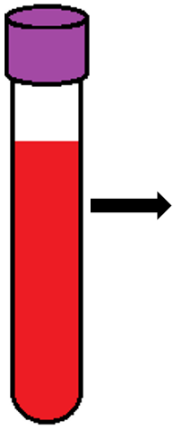
Step Three

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

Step Four

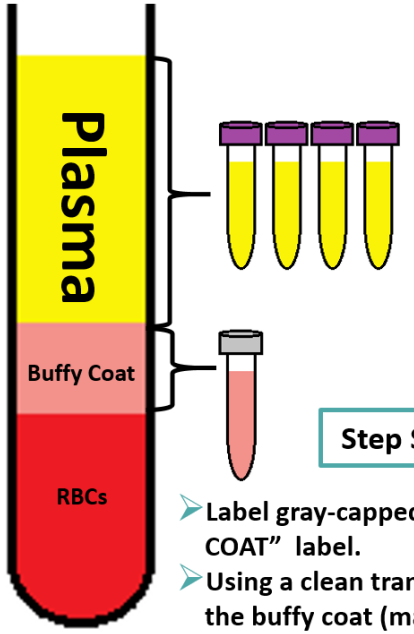
- Place thoroughly mixed tube on wet ice until centrifugation begins.

Step Five



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

Step Six



- Label purple-capped cryovials with "PLASMA" labels.
- Aliquot 1.0 ml into each cryovial.
- Store plasma aliquots upright at -80°C until shipment to NCRAD.

Step Seven

- Label gray-capped cryovial with "BUFFY COAT" label.
- Using a clean transfer pipette, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat into the cryovial
- Store buffy coat aliquot upright at -80°C until shipment to NCRAD.
- Spin, aliquot, and freeze aliquots within 1 hour of collection.

7.0 CEREBROSPINAL FLUID COLLECTION

7.1 General CSF Guidelines

- **Collect CSF Monday-Thursday only.** Do not collect CSF on Friday because **UPenn** and **NCRAD** staff will not be available to receive samples on Saturday or Sunday.
- CSF should be collected the same day as blood, following a minimum 6 hour fast.
- Begin by confirming participant consented to lumbar puncture (LP) before scheduling the procedure and again prior to performing procedure.
- 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) is allowed; however, the BioSTAC study will not reimburse sites for the cost of fluoroscopy-guided LP. Site personnel should advise the participant that use of fluoroscopy (x-rays) involves exposure to radiation.
- It is up to site clinicians to decide how to manage patients on antiplatelet therapy. For NSAIDs or aspirin or clopidogrel alone, there is no consistent evidence for increased risk. For participants on dual antiplatelet therapy or with a history of bruising on single drug therapy, the drug may be discontinued for a period before the LP and for 24 hours after LP at the discretion of the clinician.
- Ensure you have sufficient Lumbar Puncture Tray Kits and 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. Check expiration dates on all supplies, especially lidocaine.

7.2 CSF Collection Method

LPs for CSF collection should be performed using a small caliber atraumatic needle or a cutting (Quincke) needle if that is the strong preference of the clinician. **CSF samples of 2.0-2.5 mL sent to UPenn MUST be obtained via gravity flow.** It is possible to use a Sprotte needle (even 24g) for gravity collection of 2.5 mL CSF. Additional CSF may then be collected using a syringe, if that works best.

It is suggested that CSF be obtained from participants in a sitting position. Positioning is at the discretion of the person performing the LP. Alternate

needles, positions or methods (e.g., use of fluoroscopy) should be noted on the **UPenn** and/or **NCRAD** web-based sample forms (paper copy of NCRAD sample form found in Appendix C).

Collection of CSF by Gravity

After the spinal needle is placed in the intrathecal space and the stylet is withdrawn, CSF should flow freely. **If initial CSF is blood-tinged, then discard CSF until it clears. Collect 2.0-2.5 ml of CSF directly into the low protein binding CSF collection tube.**

7.3 Scheduling the LP

All LPs should be performed in the morning following a minimum 6 hour fast. CSF amyloid levels can vary depending upon the time of day the sample is collected. The LP should be rescheduled if the participant does not feel well or is febrile.

7.4 Setting up the LP

It is critical to try to optimize positioning, and usually requires an assistant. The position and needle should be recorded on the **UPenn** and **NCRAD** web-based sample forms (paper copy of NCRAD sample form found in Appendix C).

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.

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.

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.

Cleaning the Lumbar Puncture Site
The lumbar puncture site is cleaned with Povidone-Iodine Topical Solution according to best standard medical practices. Iodine is provided in a bag in the kit.

Pour enough Povidone-Iodine Topical Solution into the prep well to cover the bottom, about ¼ inch deep, and use the swabs provided to prep the skin.

Lidocaine, Syringe with Needle, Gauze Pads
Anesthesia is usually achieved within 2 minutes after injecting the lidocaine, typically 2 mL of 1% 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.

Open the iodine vial and draw 2ml into the syringe. 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.

Washcloths, Band-Aids, and Clean Up
After the person performing the lumbar puncture collects the last of the CSF, remove the needle (and introducer if a Sprotte needle was used) 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. Next, discard the LP kit following local guidelines, and dispose of sharp components in an appropriate sharps container.

7.5 Performing the LP

1. Confirm all supplies are available.

2. Label the two 2.0 ml gray-capped cryovials with provided **NCRAD** cryovial labels.

3. Measure vitals (participant lying down).

4. Collect CSF at the L3/L4 position (or adjacent position) using a Quincke or Sprotte spinal needle via gravity flow with participant in upright position (or document alternate method on the **UPenn** and/or **NCRAD** web-based sample forms (paper copy of NCRAD sample form found in Appendix C)) following these steps:

a. Collect first 1.0 – 2.0 mL of CSF for local laboratory analysis of total protein, cell count and glucose according to procedures at your Center.
b. Subsequently collect at least 2.0 ml and up to 2.5 ml CSF into one false bottom, low protein binding CSF collection tube **by gravity**.
    i. Note: 2.5 ml filling volume corresponds to filling up to the red mark on the tube in the following picture:

Each sample should be inspected for hemolysis. Do not send CSF samples which appear reddish in color to **UPenn**. Instead, collect additional clear, non-hemolytic CSF into a new false bottom, low protein binding CSF collection tube.

c. Label the 2.5 ml false bottom, low protein binding CSF collection tube with provided **UPenn** specimen label immediately following collection to keep the tube sterile.
d. Collect 1.0 ml CSF by gravity or aspiration into the 2.0 ml gray-capped cryovial. An additional 1.0 ml CSF sample may be collected and shipped to **NCRAD** if desired (extra gray-capped cryovials are provided in the supplemental kit).

5. Take the immediate post procedure vital signs.

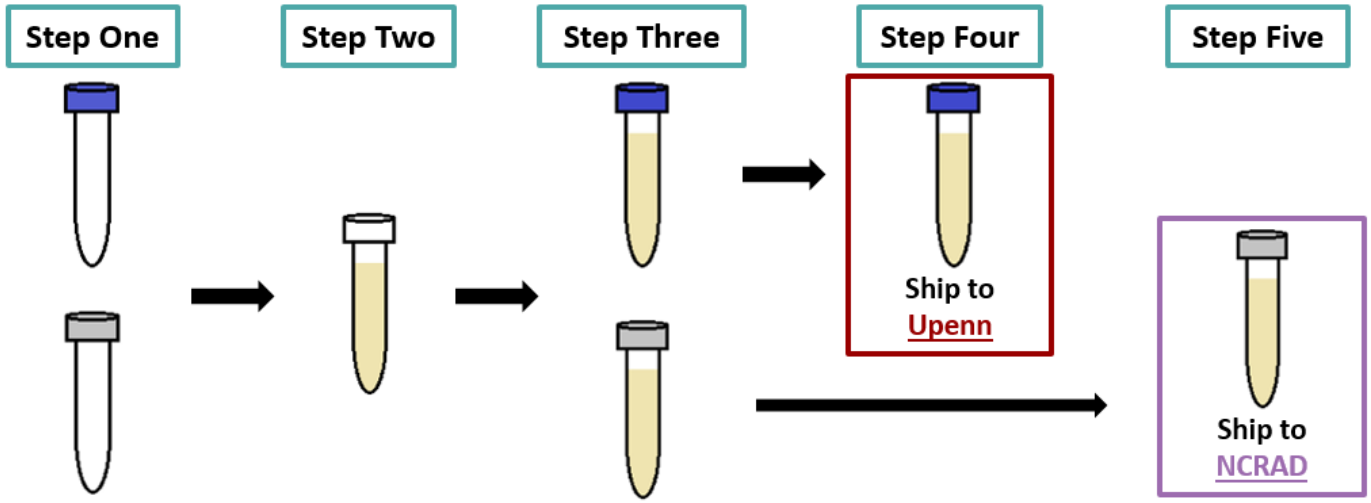
6. Provide food and drink to participant (participant may lay flat for 1-30 minutes to decrease the chance of a post-LP headache).

7. No further handling of the 2.0-2.5 ml CSF sample should be done before transport to **UPenn**. **Send the 2.0-2.5 ml CSF sample ambient to UPenn same day as collection.**

8. Store the 1.0 ml CSF sample(s) at -80°C for shipment to **NCRAD** and record time of freezing on the **NCRAD** sample form (Appendix C).

9. Enter collection data into the NACC database on day of visit.

CSF Preparation (3-3.5 ml total)



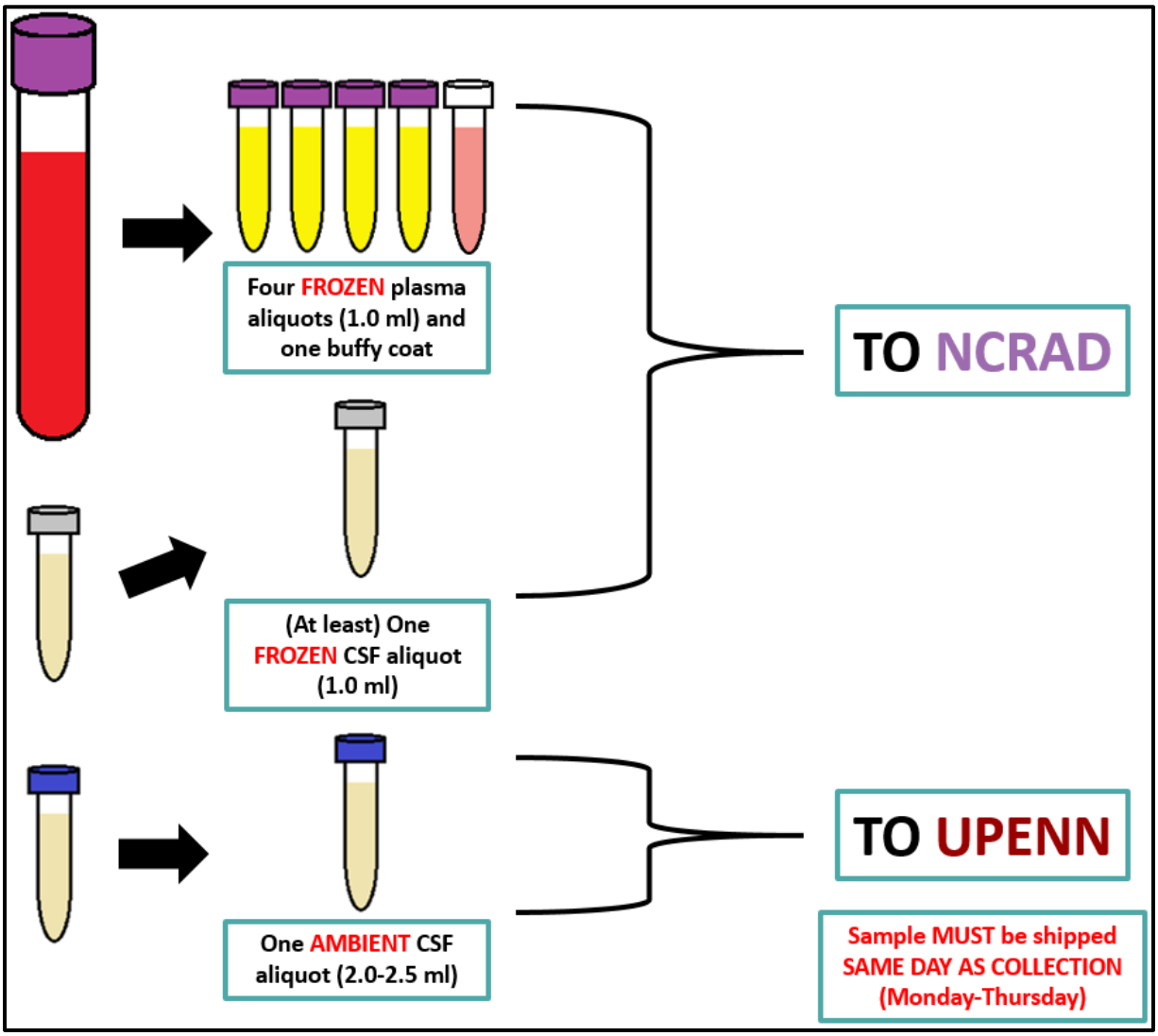
- Label CSF collection tubes with pre-printed specimen labels prior to collection.
- Collect initial 1-2 ml CSF for local lab testing.
- Collect 2.0-2.5 ml CSF into the false bottom, low protein binding CSF collection tube with UPenn label.
- Collect 1.0 ml CSF into one 2.0 ml gray-capped cryovial with NCRAD label. (Additional cryovials provided in supplemental kit to create a second 1.0 ml aliquot to send to NCRAD if desired.)
- Send 2.0-2.5 ml ambient CSF sample to UPenn SAME DAY AS COLLECTION.
- Store 1.0 ml CSF sample(s) upright at -80°C until frozen shipment to NCRAD.

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 dry ice to avoid thawing in the shipment process.

Shipping Schematic



8.1 Ambient Shipping Instructions

*****Important Note*****
AMBIENT SAMPLES ARE SHIPPED TO UPENN AND MUST BE SHIPPED MONDAY-THURSDAY ONLY!

Ambient CSF should be considered as Category B UN3373 and as such must be tripled packaged and compliant with the IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

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

- The primary receptacle (false bottom, low protein binding CSF collection tube) must be leak proof and must not contain more than 10 ml total.
- The secondary packaging (plastic canister) must be leak proof.
- 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

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 UPenn Packaging and Shipment Instructions (Ambient Shipments)

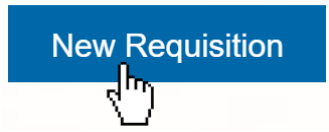
1. Place refrigerant pack in the freezer 24 hours prior to shipment.

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

3. Notify **UPenn** of shipment by completing **UPenn** web-based sample form.

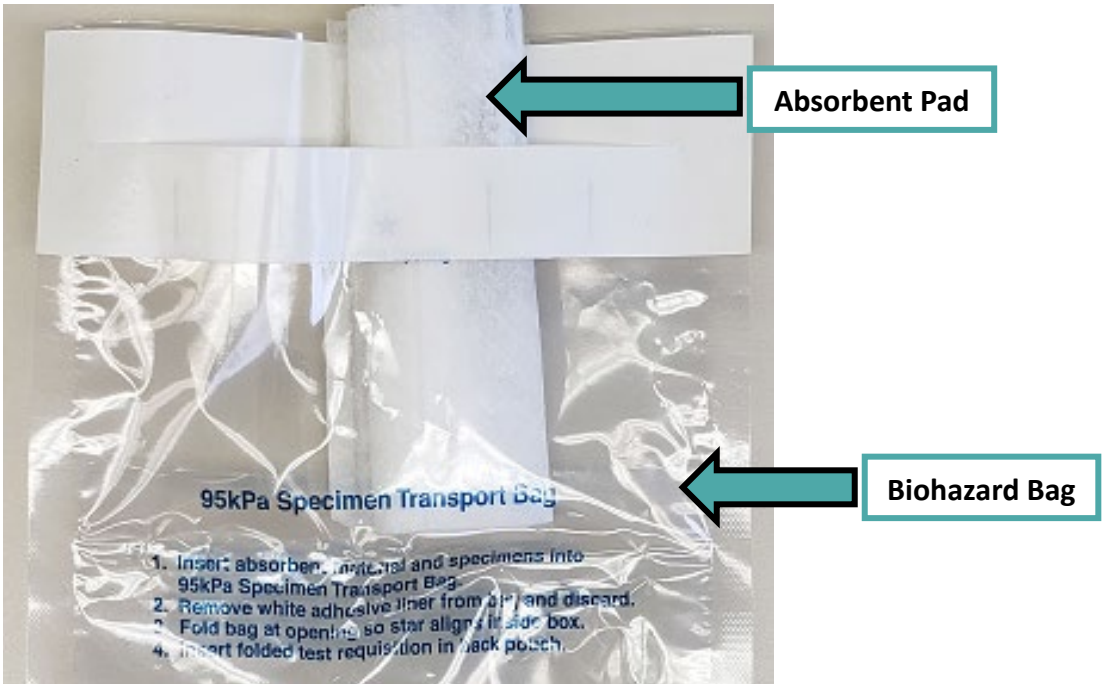
    a. Login to website at https://r3plus.pmacs.upenn.edu/ using provided credentials.

    b. Click the "New Requisition" button.



    c. Fill in the fields as follows:
        i. Patient ID: Enter subject's NACC ID.
        ii. Additional ID: enter the NCRAD Kit number that corresponds to the patient's frozen plasma, buffy coat, and CSF samples
        iii. Specimen collection date: enter the date of sample collection
        iv. Shipment tracking #: enter the Tracking Number of the ambient CSF sample's package
        v. Comment: enter any comments here if necessary.
    d. Click Submit.

4. Place filled and labeled ambient CSF sample in a slot in the absorbent pad provided, and place into the plastic biohazard bag with absorbent sheet.



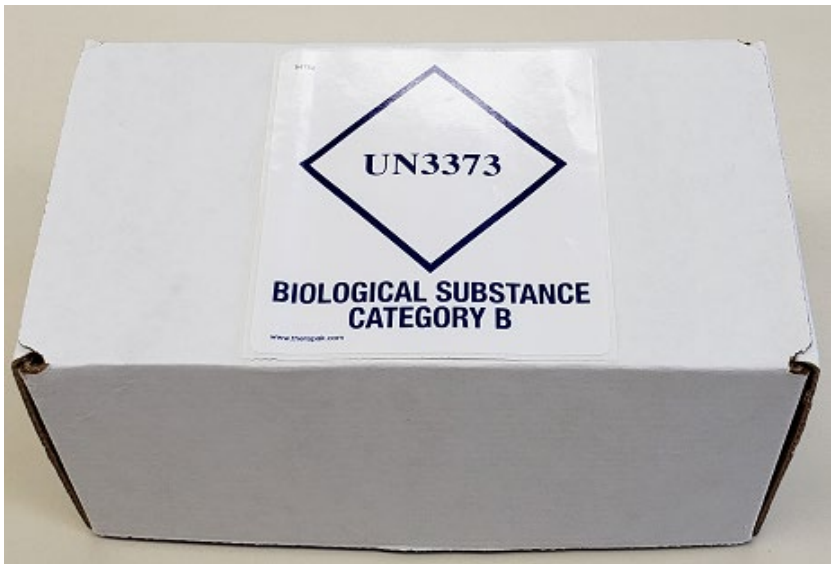
5. Remove as much air as possible from the plastic biohazard bag, and seal the bag according to the directions printed on the bag.

6. Place the refrigerant pack into the cooler on top of the filled biohazard bag.

7. Place the lid onto the cooler.

8. Place a completed list of contents card on top of the cooler lid.

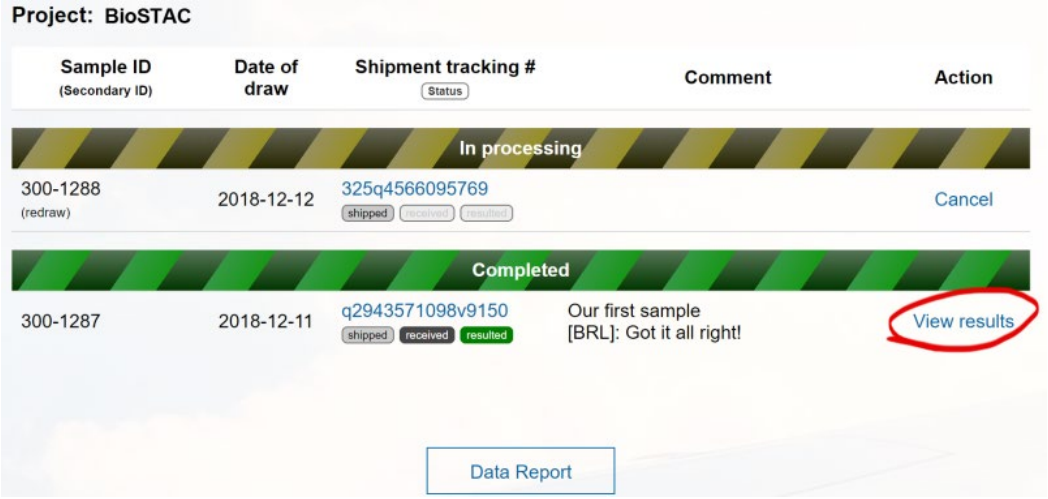
9. Close the shipping box. Label the outside of the cardboard box with the enclosed UN3373 (Biological Substance Category B) label.



10. Place the closed, labeled shipping box within a UPS Clinical Pak. **Seal the UPS Clinical Pak.**

11. Place preprinted UPS return airbill on the sealed UPS Clinical Pak.

12. Use the Dashboard on the **UPenn** web-based sample form website or UPS Tracking website to ensure the delivery occurs as scheduled and is received by **UPenn**. Results can also be viewed here.



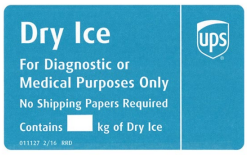
8.2 Frozen Shipping Instructions

*****Important Note*****
FROZEN SAMPLES ARE SHIPPED TO NCRAD AND 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.*

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

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



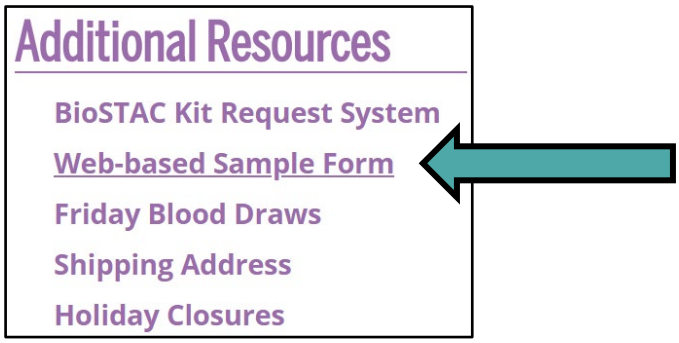
Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary 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.

8.2.1 NCRAD Packaging Instructions (Frozen Shipments)

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

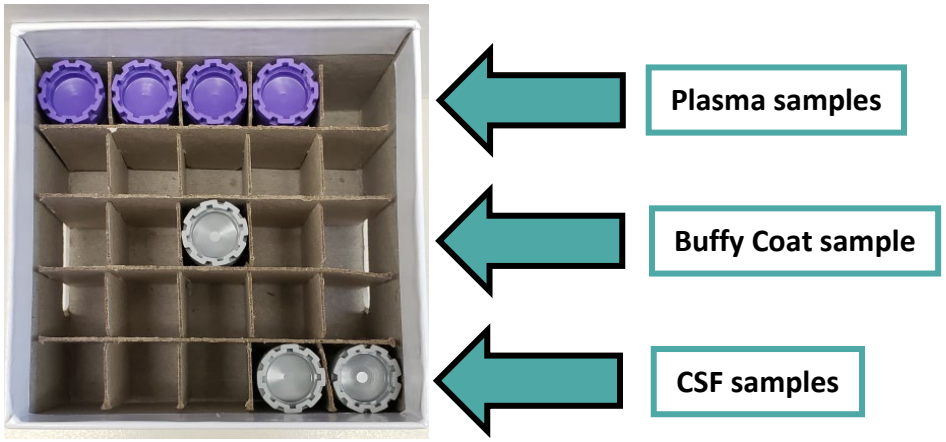
2. Notify **NCRAD** of shipment by completing **NCRAD** web-based sample form.

a. Go to NCRAD website at https://ncrad.org/resource_biostac.html.
b. Under the "Additional Resources" header on the right side of the page, select Web-based Sample Form.



c. Fill in the fields as follows:
    i. General Information: enter the sender's information.
    ii. UPS Number: enter UPS tracking number for package
    iii. Subject Information: enter the subject's information
    iv. Complete the remaining fields with the details of sample collection from the Blood/CSF Sample Processing Form (Appendix C). Keep this form for your records.
d. Add comments in the "Notes" field if necessary.
e. Click Submit Form.
f. Print a copy of the form to include with sample shipment.

3. Place all of one subject's frozen labeled aliquots of plasma, buffy coat, and CSF into one 25-slot cryovial box.
    a. A cryovial box should contain all of the specimens from the same patient, per time point.
    b. **Batch shipping should be performed every three months or when specimens from three participants accumulates, whichever is sooner.**



4. Label the outside of each cryovial box with a kit number label. Place the cryovial box containing blood derivatives and CSF in one 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. The kit number label should be placed on each cardboard cryovial box prior to inserting into the biohazard bag.

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

7. Place the three biohazard bags into the provided Styrofoam-lined shipping container on top of the dry ice. Please ensure that cryovial boxes are placed so the cryovials are upright in the shipping container.

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

9. The inner Styrofoam shipping container must contain approximately 10 lbs (or 4.5kg) of dry ice. The dry ice should entirely fill the inner box and be placed on top of the biohazard bags to ensure the frozen state of the specimens.

10. Replace the lid on the Styrofoam carton. Place the printed **NCRAD** web-based sample form in the package on top of the Styrofoam lid for each patient, and close and seal the outer cardboard shipping carton with packing tape.

11. Place UPS return airbill on the sealed box.

12. Complete the UPS Dry Ice label with the net weight of dry ice in kg (must match amount on the airbill).
    a. Do not cover any part of this label with other stickers, including pre-printed address labels.

13. Apply all provided warning labels and the completed 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.

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

15. Frozen shipments should be sent to NCRAD Monday through Wednesday to avoid shipping delays on Thursday or Friday.

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

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

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

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

SHIP ALL FROZEN SAMPLES MONDAY - WEDNESDAY ONLY!

BE AWARE OF HOLIDAYS!!

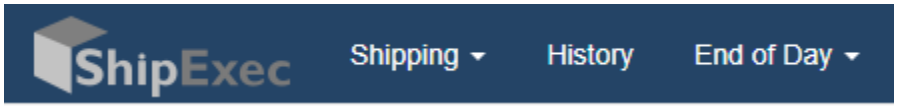
BE AWARE OF INCIPIENT INCLEMENT WEATHER THAT MAY DELAY SHIPMENT/DELIVERY OF SAMPLES

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 samples are packed with sufficient amounts of dry ice to avoid thawing in the shipment process.

8.3 NCRAD Frozen Shipping Instructions

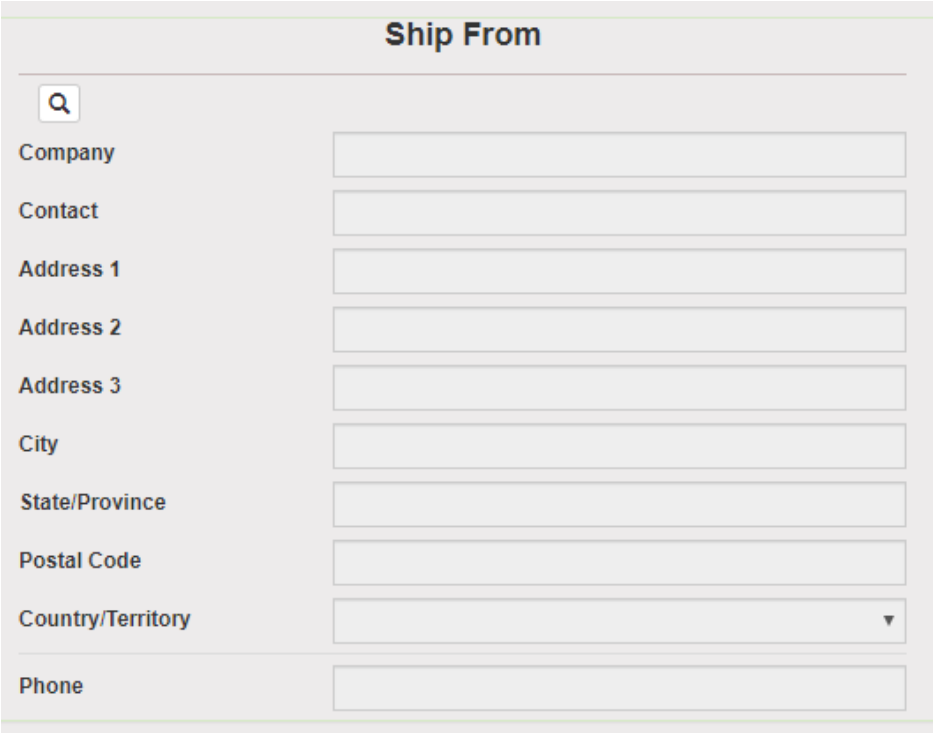
1. Log into the ShipExec Thin Client at kits.iu.edu/UPS.
    a. If a new user or contact needs access, please reach out to your study contact for access.

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.

9.0 Data Queries and Reconciliation

The **UPenn** web-based sample form and NCRAD Blood/CSF Sample Processing Form must be completed on the day that samples are collected because they capture information related to the details of sample collection and processing. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

The NACC data collection team will be collaborating with both **UPenn** and **NCRAD** to reconcile information captured in the database compared to samples received and logged at both **UPenn** and **NCRAD**. Information that appears incorrect in the NACC database will be queried through the standard system. Additional discrepancies that may be unrelated to data entry will be resolved with the Principal Investigator in a separate follow up communication.

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

- Missing samples
- Incorrect samples collected and shipped

- Damaged or incorrectly prepared samples
- Unlabeled or mislabeled samples
- Samples that are frozen and stored longer than one quarter at the site

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

Low Fat Diet Menu Suggestions

Foods to avoid prior to blood collection:

Avoid: *All Fats and nuts such as:*

- Butter
- Cream
- Bacon fat
- Lard
- All oils
- All margarine
- All nuts
- Peanut butter
- Coconut
- Whole seeds such as pumpkin and sunflower

Avoid: *All milk and dairy products such as:*

- All whole milk products
- Sour cream
- All ice cream
- All cheese
- All products containing cheese
- Milk chocolate

Avoid: *High fat prepared foods and foods naturally high in fat:*

- All red meats
- Fatty meats such as:
  - Luncheon meats
  - Organ meats
  - Bacon
- Fried foods
- Fatty fish
  - Salmon
  - Mackerel
- Buttered, au gratin, creamed, or fried vegetables
- Fried snacks such as:
  - Chips
  - Crackers
  - French Fries
- Salad dressing and mayonnaise
- Gravies and sauces
- Baked goods and frosting

Appendix C

Blood/CSF Sample Processing Form

Study: BioSTAC

Site ID: _____ **NACC ID:** NACC_____

Sex: ☐ M ☐ F **Year of Birth:** _____

Kit #: KIT BARCODE

Date subject last ate: _____[MMDDYY] **Time subject last ate:** _____ [HHMM]

Blood Collection:

Date of draw:	_____[MMDDYY]
Time of draw:	_____ [HHMM]
Original volume drawn:	_____ ml
Time spin started:	_____ [HHMM]
Duration of centrifuge:	_____ mins
Temp of centrifuge:	_____ °C
Rate of centrifuge:	_____ x g
Time aliquoted:	_____ [HHMM]
# of 1.0 ml plasma aliquots created:	_____
Time aliquots frozen:	_____ [HHMM]
Storage temperature in freezer:	_____ °C
Buffy coat aliquot created:	☐ Yes ☐ No

Ambient CSF Sample Collection:

Date of draw:	_____[MMDDYY]
Time of draw:	_____ [HHMM]
Collection method:	☐ Gravitational
Needle Used:	☐ Quincke ☐ 22g Sprotte ☐ 24g Sprotte
Volume of ambient CSF sample:	_____ ml
Ambient CSF sample barcode (6 digits):	_____

Frozen CSF Sample Collection:

Collection method:	☐ Gravitational ☐ Aspiration
# of 1.0 ml CSF aliquots created for NCRAD:	_____
(if spun) Time spin started:	_____ [HHMM] ☐ N/A
(if spun) Duration of centrifuge:	_____ mins ☐ N/A
(if spun) Temp. of centrifuge:	_____ °C ☐ N/A
(if spun) Rate of centrifuge:	_____ x g ☐ N/A
Time aliquoted:	_____ [HHMM]
Time aliquots frozen:	_____ [HHMM]
Storage temperature in freezer:	_____ °C

Notes:

_____