NAPS CONSORTIUM
For REM Sleep Behavior Disorder

Manual of Procedures

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

North American Prodromal Synucleinopathy (NAPS)

Biospecimen Collection, Processing, and Shipment Manual

Version January 2021

Manual of Procedures Version 01.05.21 Summary of Changes

Section	Change
Throughout	Addition of Visit 3 details

Table of Contents

1.0 Abbreviations

AD	Alzheimer's Disease
BL	Baseline Visit
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
GUID	Globally Unique Identifier
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
LP	Lumbar Puncture
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PHI	Protected Health Information
PK	Pharmacokinetics
RBCs	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

2.0 Purpose

The purpose of this manual is to provide NAPS 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 NAPS study visits. It includes instructions for biospecimen submission to the National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD) located at Indiana University. The following samples may be collected at each study visit:

- Plasma
- Buffy Coat (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 NCRAD.

These procedures are relevant to all study personnel responsible for processing blood specimens to be submitted to NCRAD for the NAPS protocols.

3.0 NCRAD Information

3.1 NCRAD Contacts

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

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

General NCRAD Contact Information
Phone: 1-800-526-2839
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: www.ncrad.org
NAPS Study Specific Webpage: https://ncrad.org/resource_naps.html

Milena Petkov, BS, CCRP, Study Coordinator
Phone: 317-278-1228
Email: mipetkov@iu.edu

Sample Shipment Mailing Address
NCRAD
Indiana University School of Medicine
351 W. 10th Street
TK-217
Indianapolis, IN 46202

3.2 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**.

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

3.3 Holiday Schedules

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.

3.4 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 2nd, Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2nd. If at all possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University after the second week of December. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 20th by e-mailing alzstudy@iu.edu, so that they can arrange to have staff available to process incoming samples.

Please see: https://ncrad.org/holiday_closures.html for additional information.

4.0 Globally Unique Identifier (GUID)

The GUID is a subject ID that allows researchers to share data specific to a study participant, without exposing personally identifiable information. A GUID is made up of random alpha-numeric characters and does not include any PHI in the identifier. By using GUIDs in your research data, the system can associate a single research participant's genetic, imaging, and clinical assessment data even if the data was collected at different locations or throughout different studies.

To create a GUID follow these steps:

1. Create an account: https://bricsguid.nia.nih.gov/portal/jsp/login.jsp
2. Once you have an account, go to the GUID Tool – Create GUID
3. To open the 'Launch GUID Tool' you will need to have Java installed on your device

4. In order to generate a GUID, the following PHI is required (Appendix D):
    - Complete legal given (first) name of subject at birth
    - If the subject has a middle name
    - Complete legal family (last) name of subject at birth
    - Day of birth
    - Month of birth
    - Year of birth
    - Name of city/municipality in which subject was born
    - Country of birth

5.0 NCRAD Laboratory Information

5.1 Site Required Equipment

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

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

- Dry ice (about approximately 30-45 lbs per shipment)

5.2 Biospecimens Sent to NCRAD

Biospecimens collected include whole blood and CSF

	Visit 1	Visit 2	Visit 3
Plasma	X	X	X
Buffy Coat	X	X	X
CSF*	X	X	X

*CSF collection for select sites only

Whole blood will be collected in lavender-top EDTA tubes, processed locally into plasma and buffy coat fractions, aliquoted, frozen at the study site, and shipped on dry ice to NCRAD.

CSF will be collected and aliquoted locally, frozen at the study site, and then shipped on dry ice to NCRAD.

Frozen samples are to be submitted according to the shipping methods outlined in Section 10.1. Guidelines for the processing, storage location, and timing of sample collection are listed in the tables below.

5.3 Biospecimen Collection Charts

5.3.1 Biospecimen Collection for Visits 1-3: Plasma and Buffy Coat Isolation

Sample Type	Collection Tube	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood for isolation of plasma and buffy coat **Fasting not required*	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	4	N/A	N/A	N/A
	Plasma: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	13	PLASMA: 1.5 ml plasma aliquots per 2.0 ml cryovial	Up to 14	Frozen
	Buffy Coat:	4	BUFFY COAT:	Up to 4*	Frozen

	2.0 ml cryovial		0.75 ml buffy coat aliquot		

*Sites may elect to keep 1-2 buffy coats per subject locally

5.3.3 Biospecimen Collection for CSF

Sample Type	Collection Tube	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
CSF ***Fasting required***	Sterile Containers (20-30 ml CSF)	22	CSF: 1.5 ml CSF aliquots per 2.0 ml orange cryovial	Up to 20	Frozen

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

6.0 Specimen Collection Kits, Shipping Kits, and Supplies

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD, the Blood Supplemental Supply Kit, and the Frozen Shipment Kit; 2) CSF collection kits including Lumbar Puncture (LP) trays, the CSF Supplemental Supply Kit and the CSF Shipping Supply Kit; and 3) clinical lab supplies (with the exception of dry ice and equipment supplies listed in Section 5.1). These materials include blood tubes, pipettes, LP trays (when applicable), boxes for plasma/buffy coat/PK/CSF aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, NAPS ID Labels, Collection and Aliquot Tube Labels will all be provided by NCRAD. Details regarding the blood and CSF Kits are found in this Manual of Procedures. Collection and Aliquot Tube Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 7.1.

6.1 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.*

NAPS Blood Collection Kit- Visit 1

Quantity	Blood Collection Kit Components
4	EDTA Lavender Top Blood Collection Tube (10 ml)
1	50-ml conical polypropylene tube-individually wrapped
13	Cryovial (2.0 ml) with lavender cap
4	Cryovial (2.0 ml) with clear cap
1	Cryovial (2.0 ml) with blue cap
2	Disposable graduated transfer pipette
18	Pre-printed Collection and Aliquot Tube Label
2	Pre-printed Kit Number Label
4	Labels for Handwritten Site and NAPS ID
1	Microcentrifuge box (25-slot)

NAPS Blood Collection Kit- Visit 2

Quantity	Blood Collection Kit Components
4	EDTA Lavender Top Blood Collection Tube (10 mL)
1	50-ml conical polypropylene tube-individually wrapped
3	Cryovial (2.0 mL) with lavender cap
4	Cryovial (2.0 ml) with clear cap
1	Cryovial (2.0 mL) with blue cap
2	Disposable graduated transfer pipette
18	Pre-printed Collection and Aliquot Tube Label
2	Pre-printed Kit Number Label
4	Labels for Handwritten Site and NAPS ID
1	Microcentrifuge box (25-slot)

NAPS Blood Collection Kit- Visit 3

Quantity	Blood Collection Kit Components
4	EDTA Lavender Top Blood Collection Tube (10 mL)
1	50-ml conical polypropylene tube-individually wrapped
3	Cryovial (2.0 mL) with lavender cap
4	Cryovial (2.0 ml) with clear cap
1	Cryovial (2.0 mL) with blue cap
2	Disposable graduated transfer pipette
18	Pre-printed Collection and Aliquot Tube Label
2	Pre-printed Kit Number Label
4	Labels for Handwritten Site and NAPS ID
1	Microcentrifuge box (25-slot)

NAPS CSF Kit

Quantity	CSF Kit Components
20	Cryovial tube (2.0 ml) with orange cap
1	Cryovial tube (2.0 ml) with yellow cap
1	Cryovial tube (2.0 ml) with blue cap
2	50-ml conical polypropylene tube-individually wrapped
21	Pre-printed CSF collection and Aliquot Tube Label
2	Pre-printed Kit Number label

NAPS LP Kit

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

NAPS Supplemental CSF Kit

Quantity	CSF Supplemental Supply Kit Components
5	50-ml conical polypropylene tube-individually wrapped
50	Cryovial tube (2.0 ml) with orange cap
5	Cryovial tube (2.0 ml) with blue cap
5	Cryovial tube (2.0 ml) with yellow cap
5	3 ½" × 22 (or 24) Sprotte needle with Introducer (90mm)

NAPS Blood Supplemental Supply Kit

Quantity	Blood-Based Supplemental Supply Kit Components
5	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
5	50-ml conical polypropylene tube-individually wrapped
10	Cryovial tube (2.0 ml) with lavender cap
10	Cryovial tube (2.0 ml) with blue cap

10	Cryovial tube (2.0 ml) with clear cap
20	Disposable graduated transfer pipette
20	Labels for handwritten Site and NAPS ID
5	Microcentrifuge box (25-slot)
3	Pre-printed airbills/shipping labels/plastic protective pouches
3	Warning Label Package
5	Small Biohazard bag with absorbent sheet
2	Fine Point Marker Pens

NAPS Frozen Shipping Kit

Quantity	Frozen Shipping Kit Components
8	Plastic Biohazard bag with absorbent sheet (small)
1	FedEx return airbill
1	Shipping box/Styrofoam container
1	Warning label packet with dry ice sticker

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module.
By Request	Microcentrifuge box (25-slot)
By Request	Sprotte needle, 22 or 24 gauge X 3.5" (90mm)
By Request	Cryovial tube (2.0 ml) with lavender cap
By Request	Cryovial tube (2.0 ml) with orange cap
By Request	Cryovial tube (2.0 ml) with yellow cap
By Request	Cryovial tube (2.0 ml) with blue cap
By Request	Cryovial tube (2.0 ml) with clear cap
By Request	50-ml conical polypropylene tube-individually wrapped
By Request	15-ml conical polypropylene tube-individually wrapped
By Request	FedEx return airbill
By Request	Shipping container for dry ice shipment
By Request	Plastic biohazard bag with absorbent sheet (small)
By Request	Disposable graduated transfer pipette
By Request	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
By Request	Warning label packet
By Request	UN3373 label
By Request	Biohazard label
By Request	Dry ice shipping label
By Request	Fine Point Marker Pens
By Request	Site and NAPS ID Labels

6.2 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 or supplies expire so you are prepared for study visits. Please go to kits.iu.edu/NAPS to request additional kits and follow the prompts to request the desired supplies. Options include ordering a specific number of kits; we are also including the option of simply ordering the desired amount of extra supplies.

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

7.0 Blood Collection and Processing Procedures

7.1 Labeling Samples

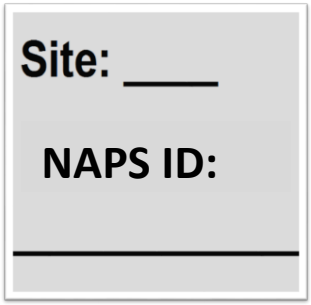
*****Important Note*****

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. Please note that the centrifuge may take 30 minutes to cool, so please plan accordingly. Draw blood in the following order:

1. **EDTA (Lavender-Top) Blood Collection Tubes (10 ml) for Plasma and Buffy Coat for NCRAD**
2. **Any other tubes for internal studies (optional)**

Label Type Summary

1. Kit Number Label
2. Site and NAPS ID Label
3. Collection and Aliquot Tube Label

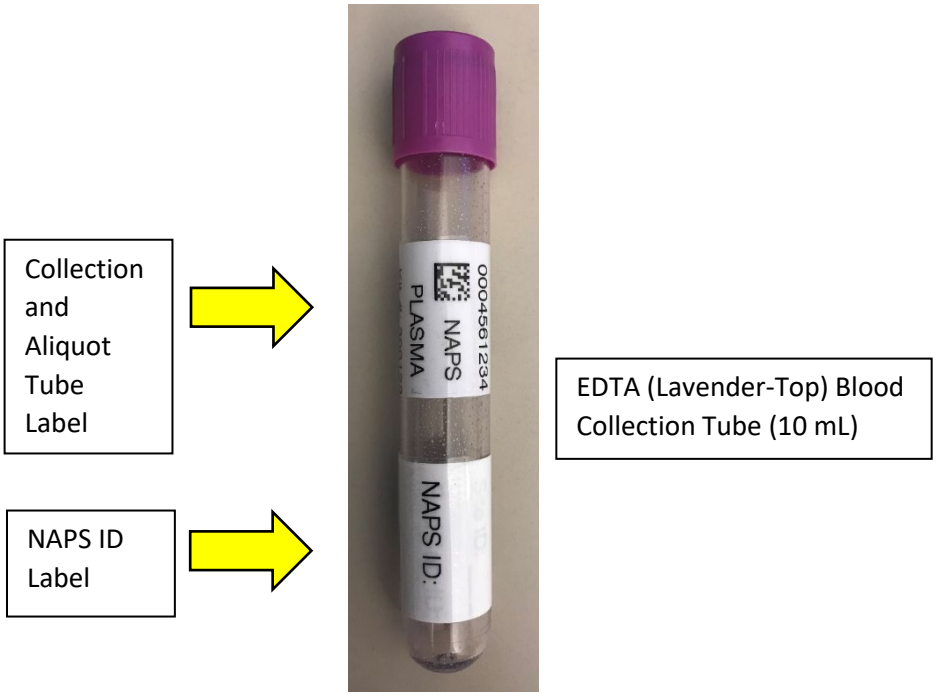


The **Kit Number Labels** do not indicate a specimen type, but are affixed on the Biological Sample and Shipment Notification Forms and on specific packing materials.

The **NAPS ID Labels** are placed on all collection tubes, both blood and CSF.

The **Collection and Aliquot Tube Labels** for blood derivatives and CSF are placed on all collection and aliquot tubes.

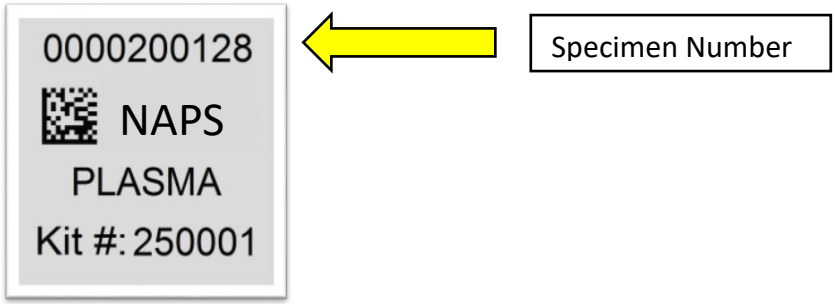
Important Note
Each collection tube will contain two labels: the Collection and Aliquot Tube Label and the NAPS ID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten NAPS ID Label.



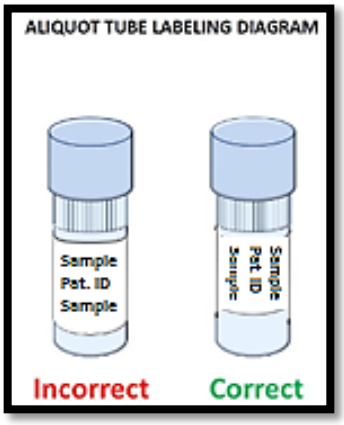
In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place blood collection and aliquot labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing, or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.

- Place cryovials in numerical order based on the specimen number, located at the top of the label. This ensures that no aliquot is misplaced or lost during the shipment process



- Using a fine point marker, fill-in and place the NAPS ID Labels on the collection tubes only (EDTA) **BEFORE** sample collection, processing, or freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place Site and NAPS ID labels on any cryovials.
- The Collection and Aliquot Tube Labels contain a 2D barcode on the left hand side of the label. Place this barcode toward the tube cap.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see 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.



- 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.

7.2 Video List

The following training videos are available to assist you with the specimen processing, aliquoting, and shipping processes. The videos are available at: https://ncrad.org/resource_naps.html

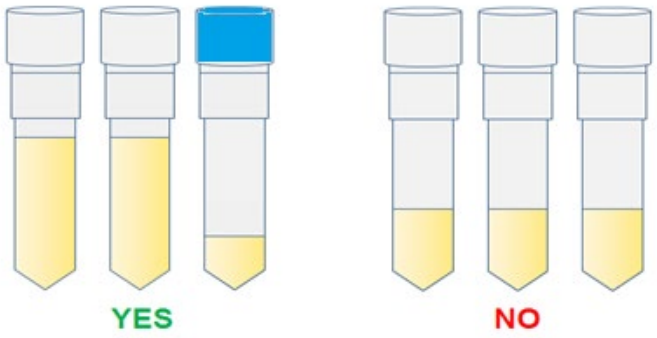
- NAPS MOP Training
- Plasma and Buffy Coat Processing and Aliquoting
- Frozen Shipping

7.3 Filling Aliquot Tubes (Plasma and CSF)

In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each cryovial should be filled to the assigned volume with the respective biological material after processing is completed (refer to detailed processing instructions for average yield per sample).

Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample.

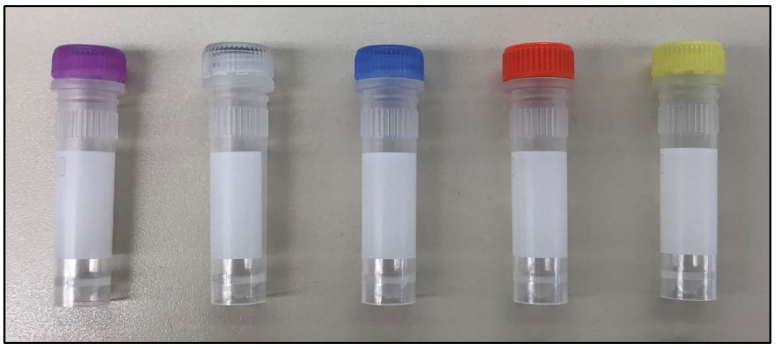
Aliquot the remaining biologic material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 3.6 ml of sample is obtained, you should fill 2 cryovial tubes each with 1.5 ml, and one additional cryovial tube with the remaining 0.6 ml for plasma. For CSF, fill up to 20 cryovials with 1.5 ml with any residual in a blue-cap cryovial.



Please note: It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume (anything under 1.5 ml for blood and anything under 1.5 ml for CSF). Please record the specimen number and volume of the residual aliquot on the Biological Sample and Notification Form.

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

Cap Color	Sample Type
Lavender Cap	Plasma/Plasma-PK
Clear Cap	Buffy Coat
Blue Cap	Residual
Orange Cap	CSF
Yellow Cap	CSF to Local Lab



7.4 EDTA (Lavender-top) Blood Collection Tubes (10 ml) for Plasma and Buffy Coat

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

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

2. Place completed NAPS ID Label and pre-printed "**PLASMA**" Collection and Aliquot Tube Label on the four lavender-top EDTA tubes. Place pre-printed "**PLASMA**" Collection and Aliquot Tube Labels on the 13 2.0 ml cryovial tubes with lavender caps and on the one 2.0 ml blue cap cryovial. Place pre-printed "**BUFFY COAT**" Collection and Aliquot Tube Label on the four 2 ml cryovials with clear caps.

3. Please ensure that aliquots are kept in numerical order (by specimen number) throughout the aliquoting and shipping process.

4. Using a blood collection set and a holder, collect blood into the **EDTA (Lavender-Top) Blood Collection Tubes (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.

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.

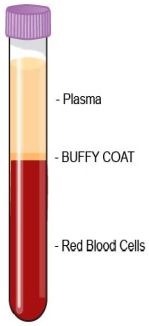
6. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tubes 8-10 times.**

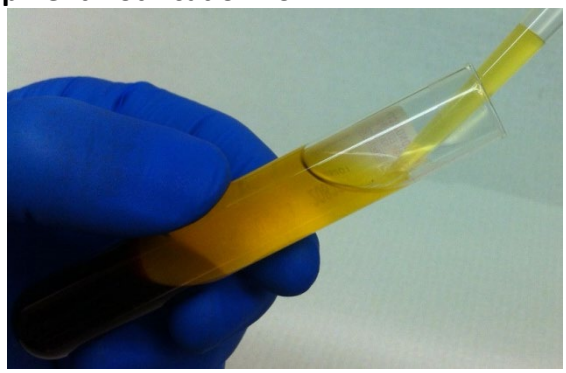
7. **CRITICAL STEP: Immediately after inverting the EDTA tubes, place on wet ice until centrifugation begins.**

   a. Preferably within 30 minutes of blood collection, centrifuge balanced tubes for 10 minutes at 2000 RCF (x g) at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM.**
   b. Equivalent rpm for spin at 2000 x g.
   c. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
   d. Plasma samples need to be spun, aliquoted, and placed upright in the freezer within 2 hours from the time of collection.
   e. Record time aliquoted on the Biological Sample and Shipment Notification Form.

8. Remove the plasma, being careful not to agitate the packed red blood cells at the bottom of the collection tube. Tilt the tube and placing the disposable pipette tip along the lower side of the wall without touching the pellet (buffy

coat) so that plasma is not contaminated (see below). Transfer plasma from all four EDTA tubes into the 50 ml conical tube and gently invert 3 times. Aliquot 1.5 ml per cryovial (13 vials with 1.5 ml each). Be sure to only place **plasma** in cryovials labeled with "PLASMA" labels**.** Take caution not to disturb the red blood cells at the bottom of the tube. If there is extra plasma left, use the extra cryovials with blue cap provided for another <1.5 ml aliquot of plasma. **If a residual aliquot (<1.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**





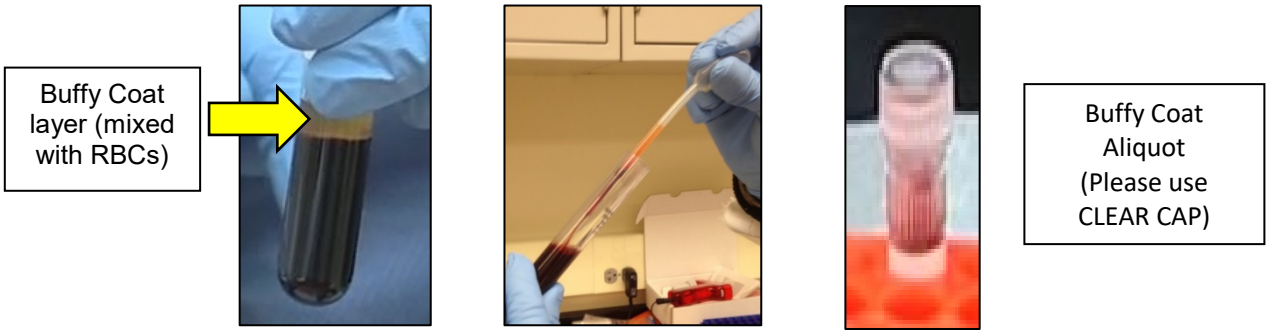
NOTE: When pipetting plasma from the plasma tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

Up to 14 cryovials possible: 13 lavender top and one blue top

9. Place the labeled cryovials in the cryobox 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 Biological Sample and Shipment Notification Form.

10. To aliquot buffy coats for Visits 1 and 2:

a. After plasma has been removed from the EDTA (Lavender-Top) Blood Collection Tubes (10 ml), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) into labeled cryovials with clear caps using a disposable graduated micropipette. Aliquot each buffy coat into a separate cryovial. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovial with the clear cap and "BUFFY COAT" label.

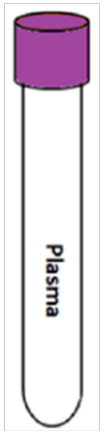


b. Dispose of collection tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.

c. Place the labeled cryovial in a cryobox and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice. Sites have the option to keep 1-2 buffy coats from each visit locally.

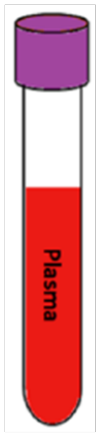
Plasma and Buffy Coat Preparation (10ml Lavender Top Tube x 4)

Step One



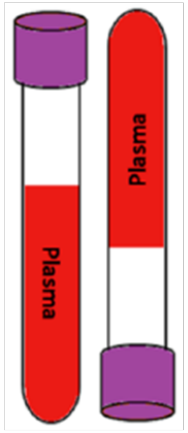
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



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

Step Three

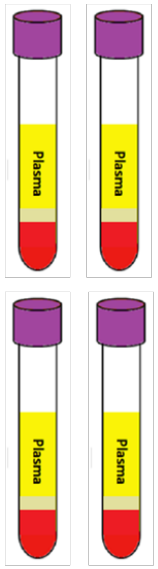


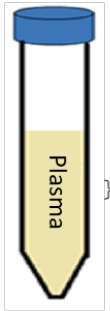
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

- Centrifuge samples at 2000 xg for 10 minutes at 4°C.
- EDTA tubes need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.

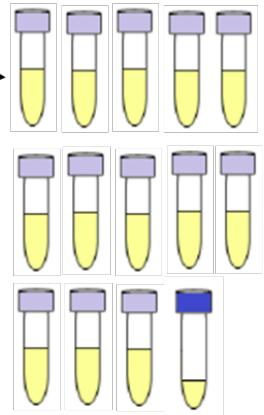
Step Five





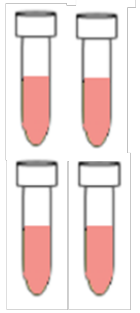
- Pool all plasma from the 4 EDTA tubes into a 50ml conical tube and invert gently 3 times to mix the plasma.

Step Six



- Label cryovial tubes with preprinted labels.
- Aliquot 1.5 ml into each cryovial tube.
- If residual aliquot is created, use blue cap to indicate volume difference and document Specimen Number on Biological Sample and Shipment Notification Form.
- Store plasma aliquots at -80°C until shipment.

Step Seven



- Label cryovials tube with preprinted label.
- Using a clean transfer pipette, collect the buffy coats (may have residual plasma and some RBCs included).
- Transfer the buffy coats into the cryovial tubes.
- Store buffy coats aliquot at -80°C until shipment.

8.0 Cerebrospinal Fluid Collection and Processing

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

CSF samples should be collected in the morning before breakfast and after an overnight fast. Only water is permitted past midnight, until lumbar puncture is completed.

There are general guidelines to follow in regards to CSF Collection.

- 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.
- LP under fluoroscopy is permitted, if needed. Site personnel should advise the subject that use of fluoroscopy (x-rays) involves exposure to radiation.
- Subjects 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. Check expiration dates on all supplies, especially lidocaine.

8.1 Scheduling the LP

All LPs should be performed between 7am and 10am local time. Availability of staff and facilities for next day blood patch should be considered when scheduling LPs. CSF amyloid levels can vary depending upon the time of day the sample is collected. It is important for the time of day of collection to remain consistent across study visits.

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

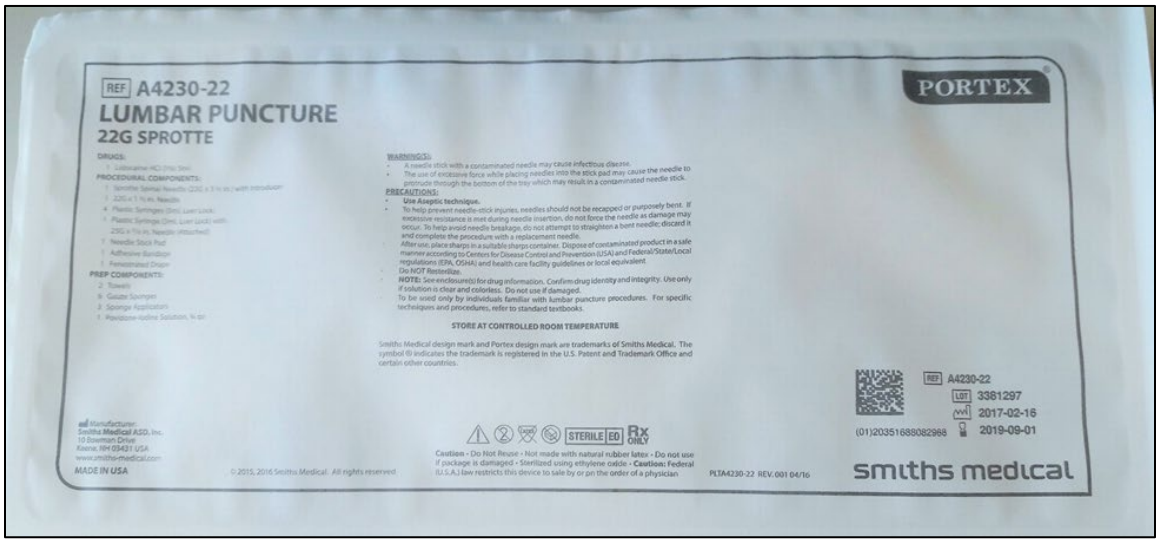
8.2 Performing the LP

The recommended position is sitting. The same position should be used at follow-up LPs when possible. 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.

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 which contains the 22 gauge Sprotte Needle with Introducer

Interior of LP Tray Provided by NCRAD

Close up of Sprotte Spinal Needle (22 gauge x 3 ½ in.) with Introducer

(24 gauge is equivalent but with lavender top needle)

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.

Next, 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 purpose. 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 is placed in the intrathecal space and the stylet is withdrawn, CSF should flow freely. **Discard first 1-2mls of CSF if blood tinged. If not blood tinged, collect first 1-2 mLs of CSF into a 50ml conical tube and pipette into the yellow cap cryovial for local lab. Collect 20-30 CSF total into the second 50ml conical tube.**

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 20-30 ml for CSF analysis. 20 ml is the required MINIMUM for CSF biomarker analysis. If 20 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. Next, discard the LP kit following local guidelines, and dispose of sharp components in an appropriate sharps container.

Step by Step Summary of CSF Collection Procedure

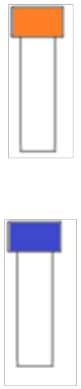
Ensure all samples collected are appropriately labeled.

1. Print CSF Sample and Shipment Notification Form.
2. Confirm all supplies, including dry ice (~10 lbs) and wet ice, are available.
3. Label the (20) orange cap cryovials and (1) blue cap cryovial with provided NAPS CSF labels. Do **NOT** open and label the 50-ml tubes that will be kept sterile to collect the CSF.

4. Pre-cool the centrifuge and pre-cool all (21) labeled cryovials (yellow cryovials will not be labeled) on wet ice. Do **NOT** pre-cool the 50-ml tubes that will be kept sterile to collect the CSF.
5. Measure vitals (participant lying down).
6. Record the time of LP and associated information on the CSF Sample and Shipment Notification Form.
7. Collect 20-30 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 50ml conical tube. If not bloody, transfer first 1-2ml into yellow cap cryovial for local lab.
   b. Collect an additional 20-30 ml CSF into the **UNLABELED-STERILE** 50-ml polypropylene tube from the "CSF Supply Kit". 20 ml is the required **MINIMUM. Collect no more than 30 ml total, including any discarded CSF and CSF kept for local lab.**
   c. If using aspiration, use **ONLY** the polypropylene syringes included in the "Lumbar Puncture Collection Kit" and transfer **DIRECTLY** into the **UNLABELED-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.
8. 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 prior to processing. Within 15 minutes of collection, centrifuge at 2000 x g for 10 min at 4°C to pellet any cellular debris.
   b. Aliquot 1.5ml into the orange-cap cryovials, being sure to not disturb the debris pellet. If a residual aliquot is created, aliquot into blue-cap cryovial. Document specimen number and volume on CSF Sample and Shipment Notification Form.
   c. Store CSF aliquots at -80°C and 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. Measure vital signs again one hour post-LP.
12. If vital signs are stable and participant feels OK one hour post-procedure, participant may sit upright, stand, and walk.
13. Enter collection data into the EDC website on day of visit.

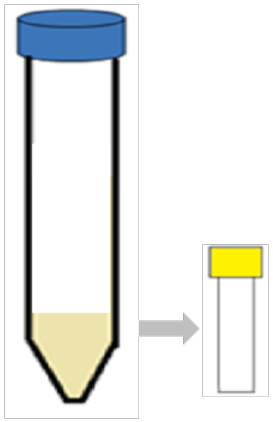
CSF Preparation (20-30 ml)

Step One



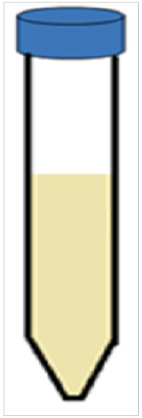
- Label tubes with pre-printed subject labels prior to collection.
- Pre-chill all cryovials on wet ice.

Step Two



- Collect initial 1-2 ml (if bloody, collect CSF until cleared of blood) into 50 ml conical tube.
- If not bloody, transfer 1-2 ml into the yellow-cap cryovial.
- Send to local lab for testing.

Step Three

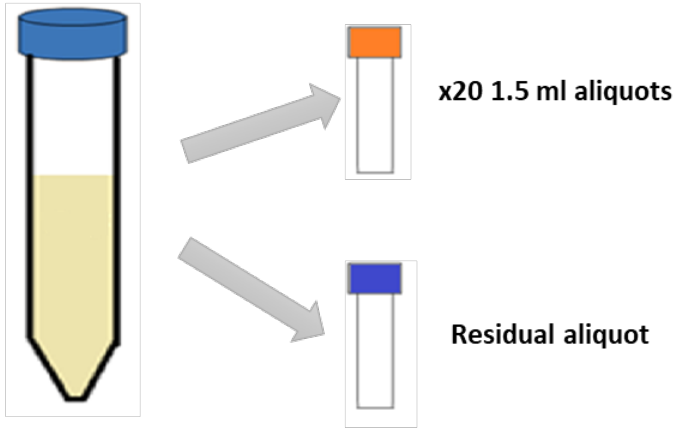


- Collect another 20-30 ml CSF into a new 50 ml sterile conical tube.

Step Four

- Place sample upright on wet ice until centrifugation begins.
- Within 15 minutes of collection, centrifuge sample at 4°C for 10 minutes at 2000xg.

Step Five



- Aliquot 1.5 ml into the orange-cap cryovials.
- If a residual aliquot is created, aliquot into blue-cap cryovial. Document specimen number and volume on CSF Sample Notification Form.
- Store CSF aliquots at -80°C until shipment.

LUMBAR PUNCTURE FOLLOW-UP PHONE CALL

This should be done the day after the lumbar puncture for all participants who had the procedure.

SUGGESTED MANAGEMENT OF POST-LUMBAR PUNCTURE HEADACHE

Classic post-lumbar puncture (low pressure) 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 NAPS LP is maximized by the use of atraumatic needles. The NAPS protocol suggests use of a 22 gauge Sprotte needle. NCRAD will provide 22 gauge Sprotte needles by default; individual sites may provide their own and use their own alternate needle type/gauge if they ensure that the needle selection and lumbar puncture technique minimize the risk of post-lumbar puncture headache. 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 and atraumatic needle with careful technique are helpful in preventing lumbar puncture headache. Having the participant refrain from exercise or strenuous activities (especially heavy lifting) 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.

Prior approval from the NAPS Coordinating Center is not necessary to perform a blood patch. However, depending on the site, local IRB approval may be required. Sites will be responsible for costs related to the performance of a blood patch.

9.0 Sample Redraws

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

If challenges arise during the blood draw process, it is advised that the phlebotomist discontinue the draw. Attempt to process and submit any blood-based specimens that have already been collected to NCRAD.

Redraws will be scheduled for samples submitted to NCRAD.

There may be situations that arise that require a patient sample to be redrawn from certain cycles/visits. At those times, NCRAD study staff will alert site coordinators that a participant sample has failed and should be redrawn. This can happen for several reasons, including insufficient blood at the time the sample was drawn, temperature storage extremes, or even shipping errors.

1. If the biospecimens at a scheduled visit are partially collected:
    a. Attempt to process and submit any samples that were able to be collected during the visit.
    b. Document difficulties on the 'Biological Sample and Shipment Notification Form' prior to submission to NCRAD.
        i. Indicate blood draw difficulties at the bottom of the 'Biological Sample and Shipment Notification Form' within the "Notes" section.
        ii. Complete the 'Biological Sample and Shipment Notification Form' with tube volume approximations and number of aliquots created.
    c. Contact a NCRAD coordinator and alert them of the challenging blood draw.
2. If the biospecimens at a scheduled visit **are not** collected:
    a. Contact the NAPS Project Manager and a NCRAD coordinator to alert them of the challenging blood draw or circumstances as to why biospecimens were not collected.
    b. Schedule participant for a re-draw visit as quickly as possible.

10.0 Packaging and Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your University, please contact NCRAD with questions and information regarding resources.

Sample Type	Processing/Aliquoting	Tubes to NCRAD	Ship
Whole blood (Lavender-Top EDTA tube) for isolation of plasma and buffy coat	Plasma: 1.5 ml plasma aliquots per 2.0 ml cryovials	Up to 14	Frozen
	Buffy Coat: 0.75 ml buffy coat aliquot per 2.0 ml cryovial	Up to 4*	Frozen
CSF	1.5 ml CSF aliquots per 2.0 ml cryovials	Up to 20	Frozen

*Sites may elect to keep 1-2 buffy coats from each visit locally.

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.

10.1 Frozen Batch Shipping Instructions

IMPORTANT!
FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY!

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

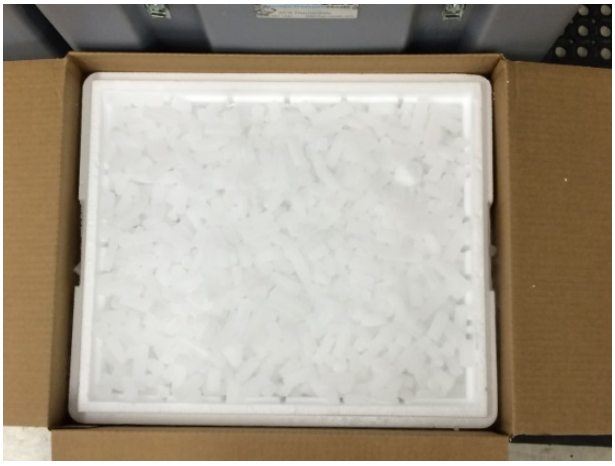
- The primary receptacle (frozen cryovials) must be leak proof and must not contain more than 1L total.
- The secondary packaging (biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (within the cryovial box containing the frozen cryovials) 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
  - ✓ Class 9 label including UN 1845, and net weight of dry ice contained

1. A sample shipment to NCRAD should be initiated when a study site has **eight (8) cryoboxes of samples or every three (3) months**, whichever is sooner.
2. Contact FedEx 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
4. Attach the Completed Biological Sample and Shipment Form to the email (Appendix B). If email is unavailable, please call NCRAD and do not ship until you have notified NCRAD coordinators of the shipment in advance.
5. Place all labeled and frozen plasma, and buffy coat aliquots in a cryobox.

6. If collecting CSF, place up to 20 CSF aliquots in a 25-slot cryobox. Place up to 14 plasma aliquots and up to four buffy coats in a separate 25-slot cryobox. Label the outside of the cryoboxes with the kit number labels and place in a clear biohazard bag. Do not remove absorbent material found in the bag and seal according to the instructions on the bag.


7. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam shipping container.
8. Place the biohazard bag into the provided Styrofoam-lined shipping container on top of the dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container. Layer dry ice and cryoboxes as necessary.
9. The inner Styrofoam shipping container must contain approximately 30-45 lbs (or ~21kg) of dry ice. The dry ice should entirely fill the inner box to ensure the frozen state of the specimens.

Full Shipping Container with
Batched Samples and Dry Ice



10. Replace the lid of the Styrofoam container. Place the completed Biological Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape. Be sure to NOT completely seal the outer cardboard box with tape, as the dry ice needs to vent.
11. Complete the FedEx return airbill with the following information:
    a. Section 1, "From": fill in your name, address, phone number, and Site FedEx Account Number.
    b. Section 2, "Your Internal Billing Reference": add any additional information required by your site.
    c. Section 6, "Special Handling and Delivery Signature Options": under "Does this shipment contain dangerous goods?" check the boxes for "Yes, Shipper's Declaration not required" and "Dry Ice". Enter the number of packages (1) x the net weight of dry ice in kg.
    d. Section 7, "Payment", check third party and bill transportation costs to the NAPS study FedEx account number.
12. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
    a. Your name and return address
    b. Net weight of dry ice in kg (must match amount on the airbill)
    c. Consignee name and address:

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

    d. 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 FedEx return airbill to the outside of the package, taking care not to overlap labels.
14. Hold packaged samples in -80C freezer until time of FedEx pick-up/drop-off.
15. Specimens should be sent to the address below via FedEx Priority Overnight. Frozen specimens should be sent Monday through Wednesday to avoid any potential shipping delays. FedEx does not replenish dry ice if shipments are delayed or held over the weekend.

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

16. Use FedEx tracking to ensure the delivery occurs as scheduled and is received by NCRAD. Please notify NCRAD by email (alzstudy@iu.edu) that a shipment has been sent and include the FedEx tracking number in your email.

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

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

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.

11.0 Data Query and Reconciliation

The Laboratory worksheets must be completed on the day that samples are collected since they capture information related to the details of the sample collection and processing. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.
[Insert other queries depending on data collection team]
Data queries or discrepancies with samples shipped and received at NCRAD may result from:

- Missing samples
- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Biological Sample and Shipment Notification Form and logged at NCRAD compared to information entered into the REDCap database.
- Samples that are frozen and stored longer than one quarter at the site
- Use of an incorrect Biological or CSF Sample and Shipment Notification Form

12.0 Appendices List

Appendix A. Rate of Centrifuge Worksheet

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. Biological Sample and Shipment Notification Form

Biological Sample and Shipment Notification Form

To: Kelley Faber Email: alzstudy@iu.edu FAX: 317-321-2003 Phone: 1-800-526-2839

General Information: **FedEx tracking #:** _____

From: _____ Date: _____

Phone: _____ Email: _____

Study: **NAPS** **Kit #:**

KIT BARCODE

Visit: Visit 1 Visit 2 Visit 3

NAPS ID: _____ **GUID:** _____

Sex: M F **Year of Birth:** _____ **CSF Collected?** Yes No

Blood Collection:

1. Date Drawn:	[MMDDYY]	2. Time of Draw:	[HHMM]
3. Last date subject ate:	[MMDDYY]	4. Last time subject ate:	[HHMM]

Blood Processing:

Plasma & Buffy Coat (Lavender-top) Tube (10 mL)		
Time spin started:		_____ [HHMM]
Duration of centrifuge:		_____ Minutes
Temp of centrifuge: ____ °C Rate of centrifuge: _____ x g		
Time aliquoted:		_____ [HHMM]
Number of 1.5 mL plasma aliquots created (lavender cap, up to 13):		
If applicable, volume of residual plasma aliquot (less than 1.5 mL in blue cap):		_____ mL
If applicable, specimen number of residual plasma aliquot (last four digits):		
Buffy coat #1 last four digits of specimen number:	Stored locally? ☐	
Buffy coat #1 volume: _____ mL	Original blood volume #1 drawn: _____ mL	
Buffy coat #2 last four digits of specimen number:	Stored locally? ☐	
Buffy coat #2 volume: _____ mL	Original blood volume #2 drawn: _____ mL	
Buffy coat #3 last four digits of specimen number:	Stored locally? ☐	
Buffy coat #3 volume: _____ mL	Original blood volume #3 drawn: _____ mL	
Buffy coat #4 last four digits of specimen number:	Stored locally? ☐	
Buffy coat #4 volume: _____ mL	Original blood volume #4 drawn: _____ mL	
Time aliquots placed in freezer:		_____ [HHMM]
Storage temperature of freezer:		_____ °C

Notes:

_____

_____

Please email or fax this from prior to the date of shipment.

Appendix C. CSF Sample and Shipment Notification Form

CSF 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 FAX: 317-321-2003 Phone: 1-800-526-2839

General Information: **FedEx tracking #:** _____

From: _____ Date: _____

Phone: _____ Email: _____

Study: NAPS **Kit #:** KIT BARCODE

Visit: Visit 1 Visit 2 Visit 3

NAPS ID: _____ **GUID:** _____ **Gauge needle used for LP:** 22G 24G

Sex: M F **Year of Birth:** _____ **CSF Collected?** Yes No

CSF Collection:

1. Date of collection: [MMDDYY]		2. Time of collection: [HHMM]	
3. Last date subject ate: [MMDDYY]		4. Last time subject ate: [HHMM]	
5. Collection process:	Gravity Method	Aspiration	

CSF Processing:

Time spin started:	_____ [HHMM]
Duration of centrifuge:	_____ Minutes
Temp of centrifuge: _____ °C	Rate of centrifuge: _____ x g
Total amount of CSF collected:	_____ mL
Time aliquoted:	_____ [HHMM]
Number of 1.5 mL CSF aliquots created (orange cap):	_____ x 1.5 mL
If applicable, volume of residual CSF aliquot (less than 1.5 mL in blue cap):	_____ mL
If applicable, specimen number of residual CSF aliquot	
Time frozen:	_____ [HHMM]
Storage temperature of freezer:	_____ °C

Notes:

_____

_____

Appendix D. GUID Demographics Form

Please be certain to collect the following demographic information to generate a Global Unique Identifier:

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