Manual of Procedures

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

A Trial to Evaluate the Efficacy and Safety of PQ912 in Patients with Early AD (VIVA-MIND) – Phase 2A

Biospecimen Collection, Processing, and Shipment Manual

Version 06.23.2021

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1.0 Abbreviations

AD	**Alzheimer's Disease**
ADCS	Alzheimer's Disease Cooperative Study
BL	Baseline Visit
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
ET	Early Termination Visit
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	Protective Health Information
RBCs	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

2.0 Purpose

The purpose of this manual is to provide VIVA-MIND 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 VIVA-MIND 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:

- Serum
- 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 VIVA-MIND protocols.

3.0 NCRAD Information

3.1 NCRAD Contacts

Tatiana Foroud, PhD, Core Leader
Email: tforoud@iu.edu

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

General NCRAD Contact Information
Phone: 1-800-526-2839
Email: alzstudy@iu.edu
Website: www.ncrad.org
VIVA-MIND Study Specific Webpage: https://ncrad.org/resource/viva-mind.html

Sample Shipment Mailing Address
NCRAD
Indiana University School of Medicine
351 W. 10th St
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. Please refer to the below schedule for the biospecimen collection schedule:

	Screening	Baseline	Week 4	Week 8	Week 16	Week 24	Early Term
Serum		X	X	X	X	X	X
Plasma		X	X	X	X	X	X
Buffy Coat		X	X	X	X	X	X
CSF	X					X	X

Whole blood is collected in two different collection tubes: lavender top EDTA tubes and plain red-top serum tubes. For phase 2a of the study, at Baseline through Week 24 visits, the lavender top EDTA tube is processed locally into plasma and buffy coat fractions, aliquoted, frozen at the study site, and then shipped to NCRAD. At Baseline through Week 24 the plain red-top serum tube is processed locally into serum fractions, aliquoted, frozen at the study site, and then shipped to NCRAD.

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

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

Frozen samples are to be submitted according to the shipping methods outlined in Section 10. 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 Screening Visit

Sample Type	Tube Type	Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
CSF Collection	Sterile Containers (15 ml CSF)	(1) 2.0 ml Sarstedt tube for Roche	CSF: 2.0 ml CSF per 2.0ml Sarstedt tube for Roche	1	Frozen
		26 cyrovial tubes (25 orange cap, 1 blue cap)	0.5 ml CSF aliquots per 2.0 ml orange cryovial; residual volume placed in 2.0 ml cryovial with blue cap	Up to 26	
		1 yellow cap cryovial tube	1-2 ml for local lab placed in 2.0 ml cryovial with yellow cap.	0 – do not return to NCRAD	N/A

5.3.2 Biospecimen Collection for Baseline and Weeks 4/8/16 Visits

Sample Type	Tube Type	Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
Whole blood for serum banking	Serum (Red-Top) Tube (10 mL)	1	N/A	N/A	N/A
	Serum: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	11	SERUM: 0.5 ml serum aliquots per 2.0 ml cryovial	Up to 11	Frozen
Whole blood for isolation of plasma	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	1	N/A	N/A	N/A

and buffy coat	Plasma: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	11	PLASMA: 0.5 ml plasma aliquots per 2.0 ml cryovial	Up to 11	Frozen
	Buffy Coat: 2.0 ml cryovial	1	BUFFY COAT: 0.75 ml buffy coat aliquot	1	Frozen

5.3.3 Biospecimen Collection for Week 24 Visit

Sample Type	Collection Tube	Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood for serum banking	Serum (Red-Top) Tube (10 mL)	1	N/A	N/A	N/A
	Serum: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	11	SERUM: 0.5 ml serum aliquots per 2.0 ml cryovial	Up to 11	Frozen
Whole blood for isolation of plasma and buffy coat	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	1	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)	11	PLASMA: 0.5 ml plasma aliquots per 2.0 ml cryovial	Up to 11	Frozen
	Buffy Coat: 2.0 ml cryovial	1	BUFFY COAT: 0.75 ml buffy coat aliquot	1	Frozen
CSF Collection	Sterile Containers (15 ml CSF)	(1) 2.0ml Sarstedt tube for Roche	CSF: 2.0 ml CSF per 2.0ml Sarstedt tube for Roche	1	Frozen

		26 cyrovial tubes (25 orange cap, 1 blue cap)	0.5 ml CSF aliquots per 2.0 ml orange cryovial; residual volume placed in 2.0 ml cryovial with blue cap	Up to 26	Frozen
		1 yellow cap cryovial tube	1-2 ml for local lab placed in 2.0 ml cryovial with yellow cap.	0 – do not return to NCRAD	N/A

5.3.4 Biospecimen Collection for Early Termination Visit

Sample Type	Collection Tube	Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood for serum banking	Serum (Red-Top) Tube (10 mL)	1	N/A	N/A	N/A
	Serum: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	11	SERUM: 0.5 ml serum aliquots per 2.0 ml cryovial	Up to 11	Frozen
Whole blood for isolation of plasma and buffy coat	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	1	N/A	N/A	N/A
	Plasma: 2.0 ml cryovials with lavender cap (residual volume	11	PLASMA: 0.5 ml plasma aliquots per 2.0 ml cryovial	Up to 11	Frozen

	placed in 2.0 ml cryovial with blue cap)				
	Buffy Coat: 2.0 ml cryovial	1	BUFFY COAT: 0.75 ml buffy coat aliquot	1	Frozen
CSF Collection	Sterile Containers (15 ml CSF)	(1) 2.0ml Sarstedt tube for Roche	CSF: 2.0 ml CSF per 2.0ml Sarstedt tube for Roche	1	Frozen
		26 cyrovial tubes (25 orange cap, 1 blue cap)	0.5 ml CSF aliquots per 2.0 ml orange cryovial; residual volume placed in 2.0 ml cryovial with blue cap	Up to 26	Frozen
		1 yellow cap cryovial tube	1-2 ml for local lab placed in 2.0 ml cryovial with yellow cap.	0 – do not send to NCRAD	N/A

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the **Biological Sample and Shipment Notification Form** (see Appendix 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, and the CSF Supplemental Supply Kit; 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 serum/plasma/buffy coat/CSF aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, Site and ADCS 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.*

VIVA-MIND Blood Collection Kit-Baseline, Weeks 4/8/16/24, ET

Quantity	Blood Collection Kit Components
1	Serum Red Top Blood Collection Tube (10 mL)
1	EDTA Lavender Top Blood Collection Tube (10 mL)
10	Cryovial tube (2.0 mL) with lavender cap
1	Cryovial tube (2.0 mL) with gray cap
10	Cryovial tube (2.0 mL) with red cap
2	Cryovial tube (2.0 mL) with blue cap
3	Disposable graduated transfer pipette
23	Pre-printed Aliquot Tube Label
4	Pre-printed Kit Number Label
3	Labels for Handwritten Site and ADCS ID
1	Microcentrifuge box (48-slot)

VIVA-MIND Blood Supplemental Supply Kit

Quantity	Blood-Based Supplemental Supply Kit Components
5	Serum (Red-Top) Blood Collection Tube (10 ml)
5	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
10	Disposable graduated transfer pipette
10	Labels for handwritten Site and ADCS ID
5	Microcentrifuge box (48-slot)
10	Plastic Biohazard bag with absorbent sheet (small)
3	Warning label packet with dry ice sticker
2	Fine point permanent marker

VIVA-MIND Frozen Shipping Kit (Batch)

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

VIVA-MIND Frozen Shipping Kit (Individual for Screening CSF)

Quantity	Frozen Shipping Kit Components
1	Shipping box/Styrofoam container
1	Warning label packet with dry ice sticker

VIVA-MIND LP Kits

***Sites must specify 22 or 24 gauge kit when ordering from NCRAD.**

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

VIVA-MIND CSF Kits Screening, W24, ET

Quantity	CSF Kit Components
2	15 ml conical polypropylene tube-individually wrapped
1	50ml conical polypropylene tube
1	Bubble wrap bag
1	Large Biohazard bag with absorbent sheet
1	2.0ml Sarstedt tube for Roche
25	Cryovial tube (2.0 ml) with orange cap
1	Cryovial tube (2.0 ml) with blue cap
1	Cryovial tube (2.0 ml) with yellow cap
3	Pre-printed Kit Number labels
27	Pre-printed Aliquot Tube Label
2	Disposable pipet
1	Microcentrifuge box (48-slot)

Supplemental CSF Kits

Quantity	CSF Supplemental Supply Kit Components
20	15ml conical polypropylene tube-individually wrapped
10	2.0ml Sarstedt tube for Roche
5	3 ½" × 22 or 24G Sprotte needle with Introducer (90mm)

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module.
By Request	Microcentrifuge box (48-slot)
By Request	Cryovial tube (2.0 ml) with lavender cap
By Request	Cryovial tube (2.0 ml) with red 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 gray cap
By Request	2.0ml Sarstedt tube for Roche
By Request	15ml conical polypropylene tube-individually wrapped
By Request	Plastic biohazard bag with absorbent sheet (small)
By Request	Disposable graduated transfer pipette
By Request	Plain Red Top Serum (Red-Top) Blood Collection Tube (10 ml)
By Request	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
By Request	UN3373 label
By Request	Biohazard label
By Request	Dry ice shipping label
By Request	Fine Point Markers
By Request	Site and ADCS 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/vivamind 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

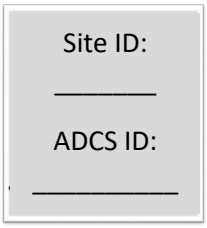
*****Important Note*****

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. **Plain Red Top Serum Blood Collection Tube (10 ml)**
2. **EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Buffy Coat and Plasma**

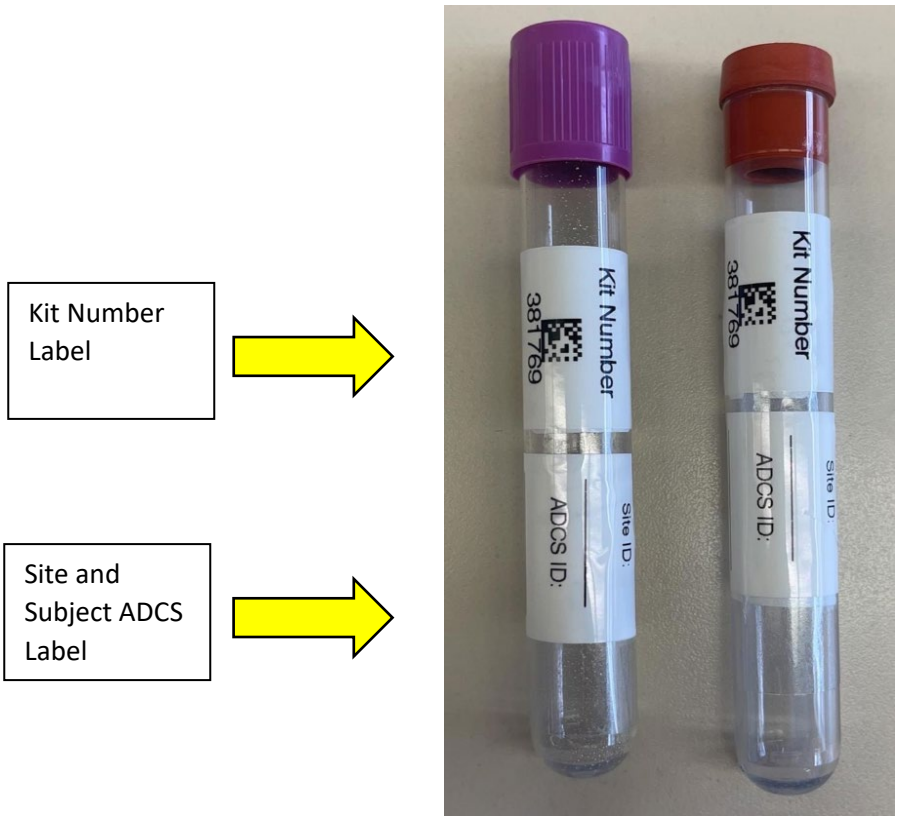
Label Type Summary

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

	The **Kit Number Labels** do not indicate a specimen type, but are affixed on the Biological Sample and Shipment Notification Forms, each collection tube label in the kit, and on the lid of the cryobox holding the samples for the kit.
	The **Site and ADCS Labels** are placed on all collection tubes, both blood and CSF.
	The **Aliquot Tube Label** is placed on each blood derivative and CSF aliquot.

Important Note

Each collection tube will contain two labels: the Kit Number Label and the Site and ADCS ID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten Site and ADCS label.



EDTA (Lavender-Top) Tube (10 mL)

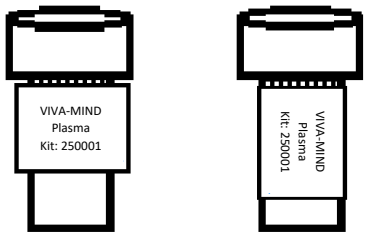
Serum (Red-Top) Tube (6 mL)

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 ADCS ID label on the collection tubes **BEFORE** sample collection or processing. This

label is in addition to the kit number label. **DO NOT** place ADCS 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.



Incorrect Correct

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

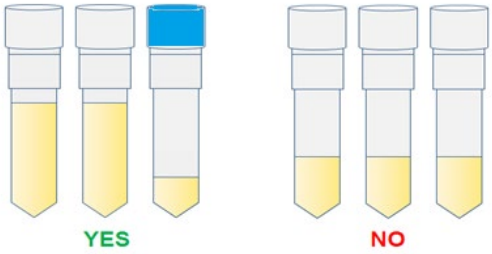
The following training videos are available to assist you with the specimen processing, aliquoting, and shipping processes. The videos are available at: https://ncrad.org/resource/viva-mind.html

- VIVA-MIND MOP Training
- Plasma and Buffy Coat Processing and Aliquoting
- Serum Processing and Aliquoting
- Frozen Shipping

7.3 Filling Aliquot Tubes (Plasma, Serum, and CSF)

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

Aliquot the remaining biologic material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 3.3 ml of sample is obtained, you should fill 6 cryovial tubes each with 0.5 ml, and one additional cryovial tube with the remaining 0.3 ml for plasma, serum, and CSF. **NOTE: 2.0ml of CSF should be aliquoted into the 2.0ml Sarstedt tube for Roche**



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

To assist in the preparation and aliquoting of samples, colored caps and cap stickers 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
Red Cap	Serum
Blue Cap	Residual
Gray Cap	Buffy Coat
Orange Cap	CSF
Yellow Cap	CSF for Local Lab
Blue Cap – Individually wrapped	Sarstedt tube for Roche

7.4 Serum (Red-top) Tube (10 mL) for Serum

Whole Blood Collection for Isolation of Serum: Serum (Red-Top) Tube (10 ml) (for processing of serum aliquots). One Red-Top tube is collected at Baselines, Weeks 4/8/16/24 and/or Early Termination, when applicable.

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

2. Place completed Site and ADCS ID Label and Collection **"SERUM"** Tube Labels on the Plain Red-Top Serum Blood Collection Tube. Place pre-printed Aliquot "**SERUM**" Tube Labels on the (10) 2.0 ml cryovial tubes with red caps and (1) 2.0 ml cryovial with blue cap (if necessary, for residual).

3. Using a blood collection set and a holder, collect blood into **Plain Red-Top Serum 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 the stopper or the end of the needle during venipuncture.

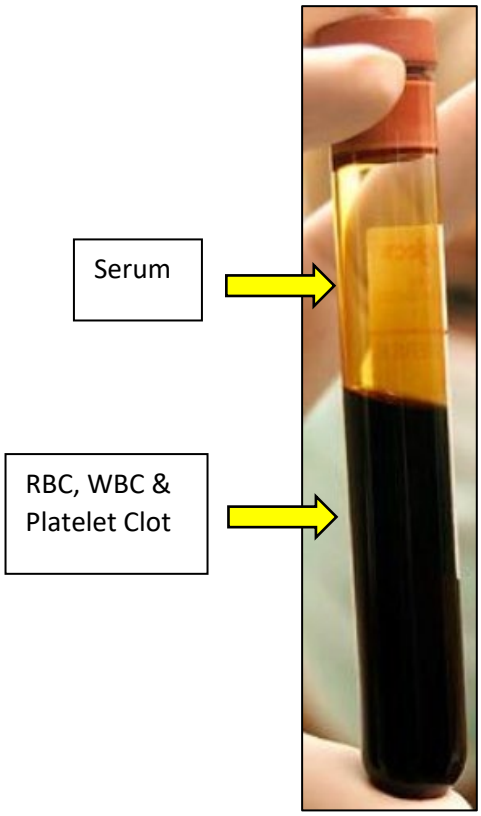
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into each tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 5 ml of blood into the tube.
   a. If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional Serum tube at this time. Process blood obtained in existing Serum tube.

5. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) each tube 5 times.**

6. **CRITICAL STEP: Allow blood to clot at room temperature by placing it upright in a vertical position in a tube rack for 30 minutes. If sample is not clotted allow it to set up to 60 minutes to clot. Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.**

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

8. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the wall. Using a disposable pipette, transfer serum into the pre-labeled cryovials with the red caps. Aliquot 0.5 ml per cryovial (total vials = up to ten with 0.5 ml each or nine with 0.5 mL and one residual with <0.5 ml). Be sure to only place **serum** in cryovials labeled with the "SERUM" label and red caps. If there is extra serum left, use 1 extra blue-cap cryovial provided for another <0.5 ml aliquot of serum and label as appropriate. **If a residual aliquot (<0.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**

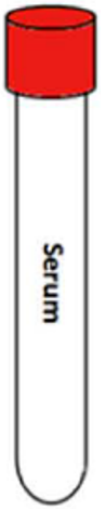


Serum Aliquots (up to 10 possible)

9. Place the labeled cryovials 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. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

Serum Preparation (10ml Red Top Tube)

Step One

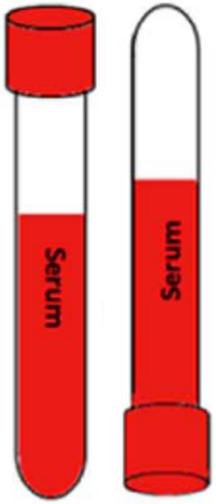


- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two

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

Step Three

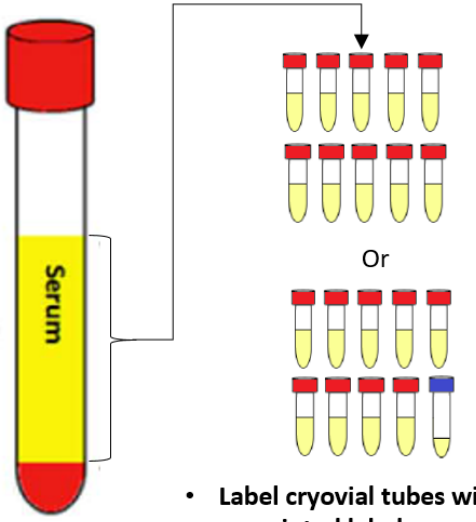


- Immediately after blood draw, invert tubes 5 times to mix samples.

Step Four

- Allow blood to clot for 30 minutes.
- Centrifuge samples at 2000 x g for 10 minutes at 4°C.
- Serum samples need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.

Step Five



- Label cryovial tubes with preprinted labels.
- Aliquot 0.5 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 serum aliquots at -80°C until shipment.

7.5 EDTA (Lavender-Top) Blood Collection Tube (10 mL) for Plasma and Buffy Coat

Whole Blood Collection for Isolation of Plasma and Buffy Coat: EDTA (Lavender-Top) Blood Collection Tube (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 Site and ADCS ID Label and pre-printed "**PLASMA**" Collection and Tube Label on the lavender-top EDTA tube. Place pre-printed "**PLASMA**" Aliquot Tube Labels on the ten (10) 2.0 ml cryovial tubes with lavender caps. Place pre-printed "**BUFFY COAT**" Collection and Aliquot Tube Label on the (1) 2 ml cryovial with a gray cap.

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 Tube (10 ml)** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:

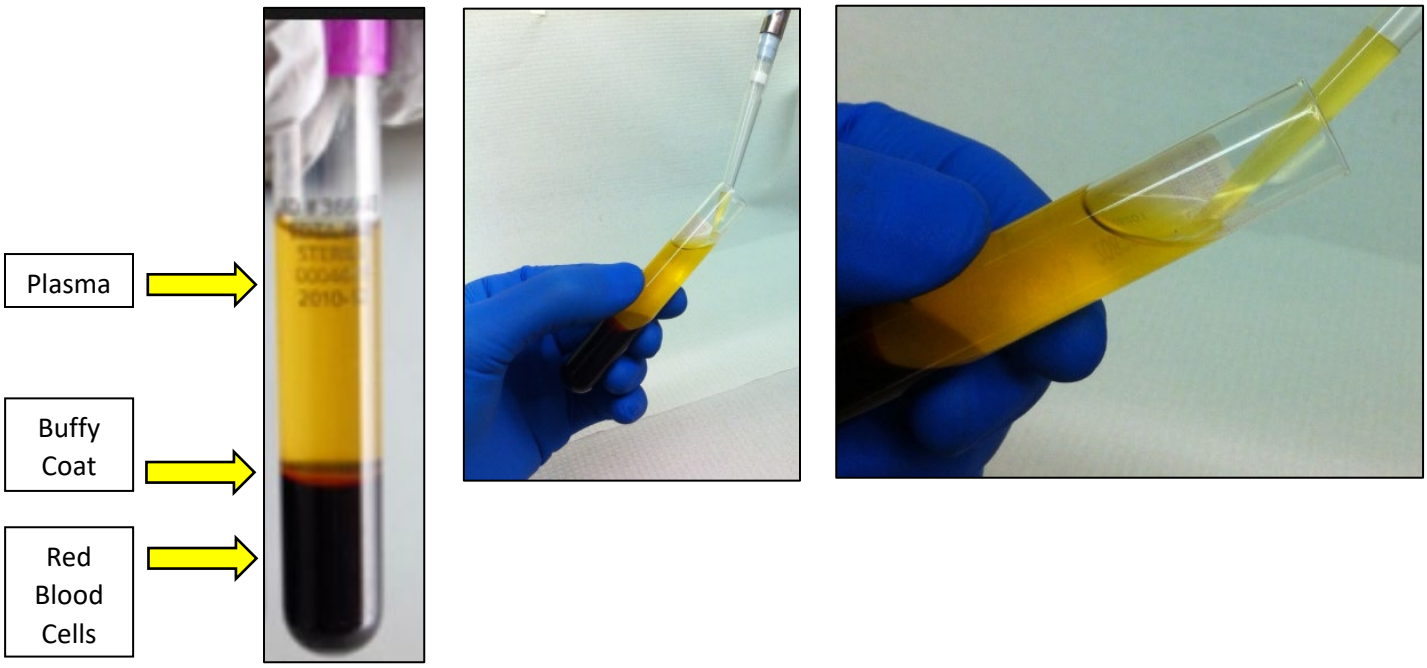
   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

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 tube 8-10 times.**

7. **CRITICAL STEP: Immediately after inverting the EDTA tube, place it 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 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 into the pre-labeled cryovials. Aliquot 0.5 ml per cryovial (up to 10 vials with 0.5 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 1 extra cryovial with blue cap provided for another <0.5 ml aliquot of plasma. **If a residual aliquot (<0.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**



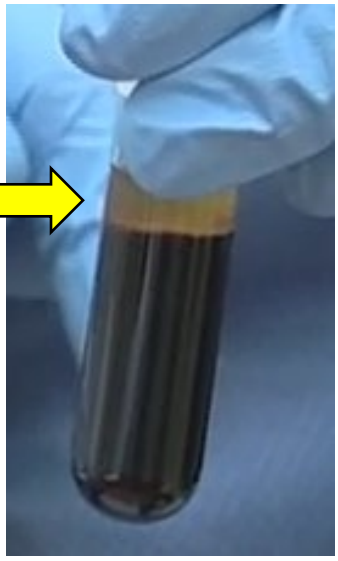
NOTE: When pipetting plasma from the plasma tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

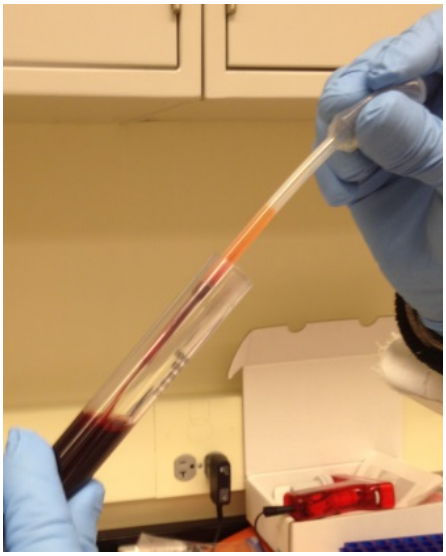
9. Place the labeled cryovials in the cryobox and place upright on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

10. After plasma has been removed from the EDTA (Lavender-Top) Blood Collection Tube (10 ml), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) into labeled cryovial with gray cap using a disposable graduated micropipette. All of the buffy coat will be 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 cryovial with the gray cap and "BUFFY COAT" label.

Up to 10 cryovials possible: nine lavender top and one blue top. Lavender top cryovials have 0.5ml plasma and blue top cryovial used if <0.5ml remains.

Buffy Coat layer (mixed with RBCs)





Buffy Coat Aliquot (Please use gray cap cryovial)

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

12. Place the labeled cryovial in a 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.

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

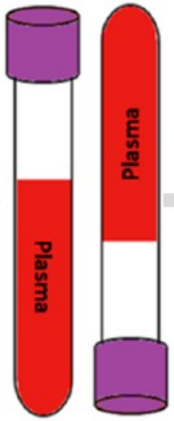
Step One

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

Step Two

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

Step Three



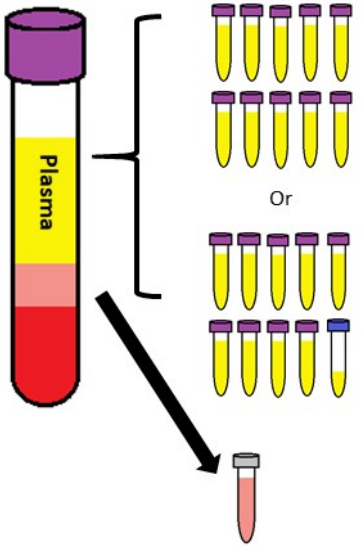
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

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

Step Five

- Preferably within 30 minutes of blood draw, centrifuge samples at 2000 x g at 4°C for 10 minutes.
- Samples need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.



Step Six

- Adhere preprinted labels to the lavender cap cryovials.
- Aliquot 0.5 ml into each cryovial tube.
- If a residual aliquot is created, document specimen number and volume on Sample Notification Form.
- Store plasma aliquots at -80°C until shipment.

Step Seven

- Adhere preprinted "BUFFY COAT" labels to the gray cap cryovial.
- Using a clean pipette tip, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat from each EDTA tube into its own cryovial tube.
- Store buffy coat aliquot at -80°C until shipment to NCRAD.

8.0 Cerebrospinal Fluid Collection and Processing Procedures

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

Fasting prior to the lumbar puncture is not required. However, efforts should be made to ensure each LP done on a participant is performed at the same time of day.

There are general guidelines to follow in regard 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

LPs can be performed in the morning or in the afternoon. 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.

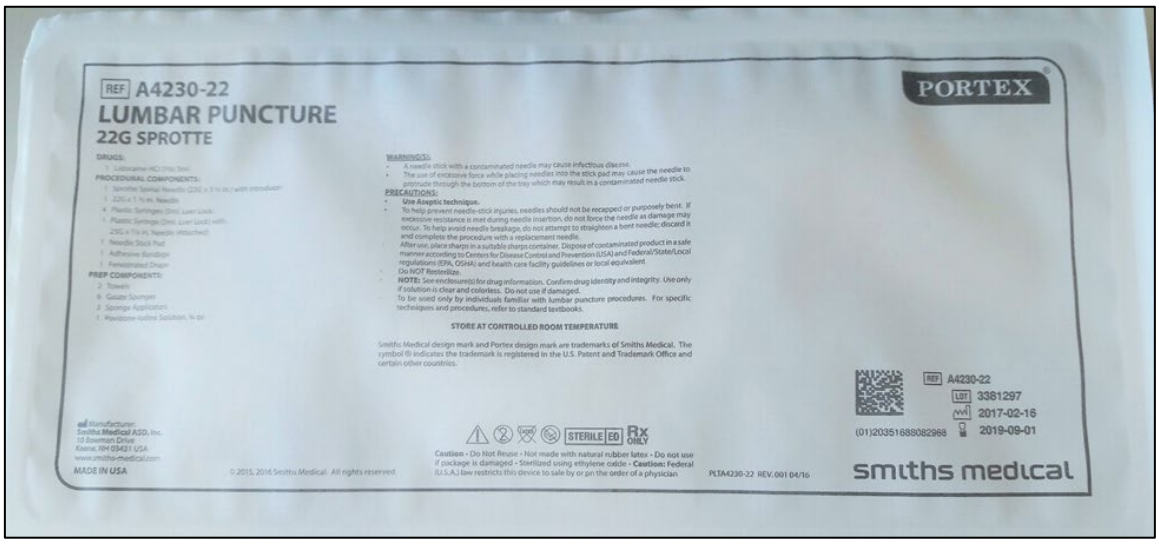
8.2 Performing the LP

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

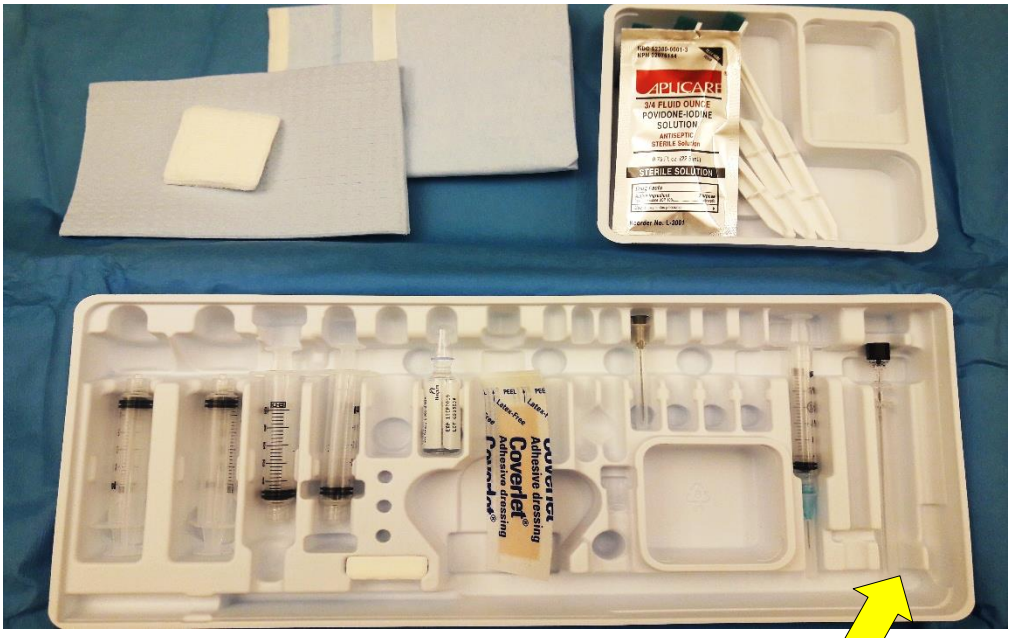
On the bedside table nearest where the person performing the lumbar puncture will sit, place a pair of sterile gloves (in their packaging) and a blue pad. Remove the contents of the lumbar puncture tray from the outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything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

Note that the 15ml conical tubes and Sarstedt collection tube for Roche, 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.

Reminder: 15 ml is the required MINIMUM for CSF biomarker analysis. If 15 ml is not obtained and provided to the NCRAD, document the reason for under-collection on the comments section of the CSF Sample and Shipment Notification Form.

Washcloths, Band-Aids, and Clean Up
After the person performing the lumbar puncture collects the last of the CSF, remove the needle and introducer and wash the Povidone-Iodine Topical Solution off the participant. A warm, wet washcloth can be used. A Band- Aid should be applied to the puncture site. 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 (25) orange cap cryovials, and (1) blue cap cryovial with provided VIVA-MIND CSF labels. Do **NOT** open and label the individually wrapped 15 ml conicals or Sarstedt tube for Roche that will be kept sterile to collect the CSF.
4. Pre-cool the centrifuge and pre-cool all labeled cryovials on wet ice. Do **NOT** pre-cool the 15 ml conicals or Sarstedt tube for Roche 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 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 15ml conical tube. If not bloody, transfer first 1-2ml into yellow cap cryovial for local lab.
    b. **Next, collect 2.0 mls directly into the 2.0ml Sarstedt tube for Roche. The tube has a "fill-to" line at the 2.0 ml mark. No processing is required for this tube – at no point is the tube centrifuged or aliquot to/from. Label the (1) 2.0ml Sarstedt tube for Roche, cap the tube and store.**
    c. Collect additional CSF (totaling 15ml including the 2.0ml-3.0ml collected initially) into the **UNLABELED-STERILE** 15ml polypropylene conicals from the "CSF Supply Kit".
    d. 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 conical 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. Preferably within 15 minutes of collection, centrifuge briefly at low speed (2000 x g, 10 min, 4°C with brake turned off) to pellet any cellular debris.
    b. Aliquot 0.5ml into the orange-cap cryovials. 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.

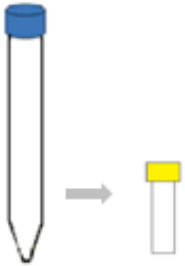
13. Enter collection data into the EDC website on day of visit.

CSF Preparation (15 ml total)

Step One

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

Step Two



- Collect initial 1-2ml (if bloody, collect CSF until cleared of blood) into 15 ml conical tube.
- If not bloody, transfer 1-2ml into the yellow cap cryovial and send to local lab for testing.

Step Three

- Next collect 2.0 ml into the 2.0ml Sarstedt tube for Roche.
- The tube has a "fill-to" line at the 2.0 ml mark.
- No processing is required for the Sarstedt tube for Roche.
- Label the Sarstedt tube for Roche, cap the tube and store.

Step Four

- Collect 15 ml total, including the 2.0-4.0 ml collected in steps two and three.

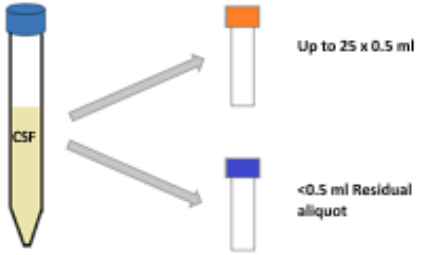
Step Five

- Place 15ml conical upright on wet ice until centrifugation begins.

Step Six

- Preferably within 15 minutes of collection, centrifuge samples at 4℃ at 2000 x g for 10 minutes.

Step Seven



- Using a clean pipette, aliquot 0.5 ml into the orange-cap cryovials, leaving the debris pellet undisturbed.
- If a residual aliquot is created, aliquot into blue-cap cryovial. Document specimen number and volume on CSF Sample Notification Form.
- Within 2 hours of CSF collection, samples need to be spun, aliquoted and in the freezer. Store at -80℃ until shipment. Record time of freezing on CSF Sample Notification Form.

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 VIVA-MIND LP is maximized by the use of atraumatic needles. 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 VIVA-MIND 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 VIVA-MIND Project Manager and a NCRAD coordinator to alert them of the challenging blood draw or circumstances as to why biospecimens were not collected.

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	Collection Tube	Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood for serum banking	Serum (Red-Top) Tube (10 mL)	1	N/A	N/A	N/A
	Serum: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	11	SERUM: 0.5 ml serum aliquots per 2.0 ml cryovial	Up to 11	Frozen
Whole blood for isolation of plasma and buffy coat	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	1	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)	11	PLASMA: 0.5 ml plasma aliquots per 2.0 ml cryovial	Up to 11	Frozen
	Buffy Coat: 2.0 ml cryovial	1	BUFFY COAT: 0.75 ml buffy coat aliquot	1	Frozen
CSF	Sterile Containers (15 ml CSF)	27	CSF: 2.0 ml CSF per 2.0ml Sarstedt tube for Roche	1	Frozen
			CSF: 0.5 ml CSF aliquots per 2.0 ml orange cryovial	Up to 26	

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 Shipping Instructions

*****Important Note*****
Screening CSF samples should be shipped every other week, Monday-Wednesday to NCRAD.

All blood samples, and Week 24 CSF samples should be shipped to NCRAD in increments of five (6) cryoboxes, or every three (3) months, whichever comes first.

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

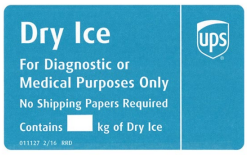
*****Important Note*****
FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY!

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

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

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

- The primary receptacle (cryovial) must be leak proof and must not contain more than 1L total.
- The secondary packaging (biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible Person
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373
  - ✓ UPS Dry Ice label and net weight of dry ice contained



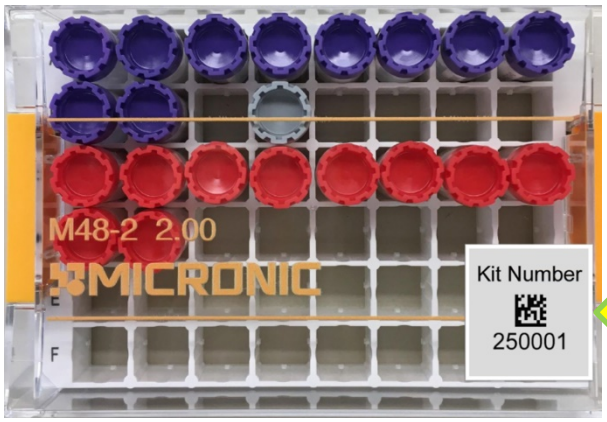
Packaging Instructions

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

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

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

a. Place plasma, buffy coat and serum samples within one cryobox per participant.



b. Place CSF samples within one cyrobox per participant.

c. Place 2.0ml Sarstedt tube for Roche in a 50ml conical. Place 50ml conical in bubble wrap tube sleeve.

4. As the samples are placed in the plastic biohazard bag, do NOT remove the absorbent material found in the bag. Seal according to the instructions on the bag.

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

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

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

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

9. Replace the lid on the Styrofoam carton. Place the completed Blood Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape.

10. Complete the UPS Dry Ice Label with the following information:
    a. Net weight of dry ice in kg (must match amount on the airbill)
    b. Do not cover any part of this label with other stickers, including preprinted address labels.

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

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

13. Specimens should be sent to the following address via UPS Next Day Air. Frozen shipments should be sent Monday through Wednesday to avoid shipping delays on Thursday or Friday.

    VIVA-MIND at NCRAD
    Indiana University School of Medicine
    351 W. 10th St. TK-217
    Indianapolis, IN 46202

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

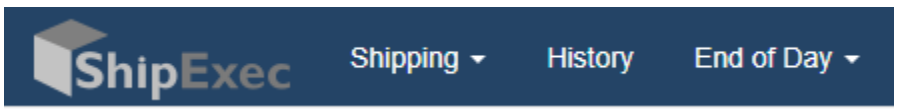
Shipping Instructions

*****Important Note*****
Screening CSF samples should be shipped every other week, Monday-Wednesday to NCRAD.

All blood samples, and Week 24 CSF samples should be shipped to NCRAD in increments of five (6) cryoboxes, or every three (3) months, whichever comes first.

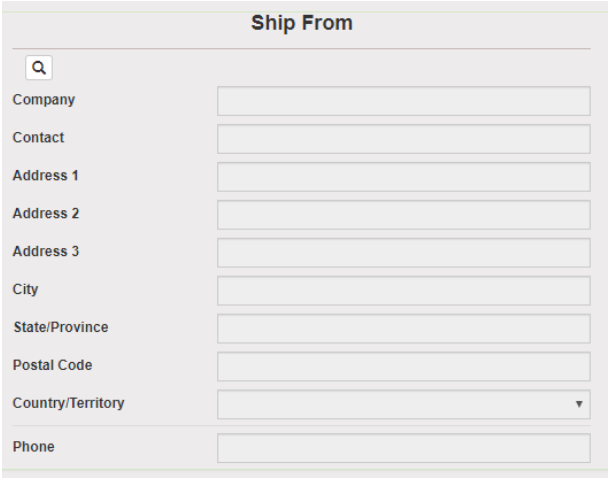
1. Log into the ShipExec Thin Client at kits.iu.edu/UPS.
   a. If a new user or contact needs access, please reach out to your study contact for access.

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. Ambient shipments
      i. Enter the total weight of your package in the “Weight” field and leave the “Dry Ice Weight” field empty.
   b. 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.
   c. 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.

11.0 Data Queries and Sample Reconciliation

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

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

- Missing samples
- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Biological Sample and Shipment Notification Form and logged at NCRAD compared to information entered into the ADCS 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

Appendix A. Rate of Centrifuge Worksheet

Rate of Centrifuge Worksheet

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

Submitter Information

Name: **Site:**

Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

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

Calculating RPM from G-Force:

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

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

Comments:

Please send this form to NCRAD Study Coordinator

alzstudy@iu.edu

Appendix B. Biological Sample and Shipment Notification Form

Biological Sample and Shipment Notification Form

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

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

General Information: **UPS tracking #:** _____

From: _____ Date: _____

Phone: _____ Email: _____

Study: VIVA-MIND **GUID:**_____ **Kit #:**

Visit: Baseline W4 W8 W16 W24 ET

KIT BARCODE

Site ID: _____ **ADCS PTID #:** _____

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

Blood Collection:

1. Date Drawn:	[MM/DD/YY]	2. Time of Draw:	[HHMM]
3. Last time subject ate:	[MM/DD/YY]	4. Last time subject ate:	[HHMM]

Blood Processing:

Serum (Red-top) Tube (10 mL)		**Plasma & Buffy Coat (Lavender-top) Tube (10 mL)**	
Time spin started:	_____ [HHMM]	Time spin started:	_____ [HHMM]
Duration of centrifuge:	_____ Minutes	Duration of centrifuge:	_____ Minutes
Temp of Centrifuge: ____ °C Rate of centrifuge: ____ x g		Temp of Centrifuge: ____ °C Rate of centrifuge: ____ x g	
Original volume drawn (1 x 10 mL tube):	_____ mL	Original volume drawn (1 x 10 mL tube):	_____ mL
Time aliquoted:	_____ [HHMM]	Time aliquoted:	_____ [HHMM]
Number of 0.5 mL serum aliquots created (red cap):		Number of 0.5 mL plasma aliquots created (lavender cap):	
If applicable, volume of residual serum aliquot (<0.5 mL in blue cap):	_____ mL	If applicable, volume of residual plasma aliquot (<0.5 mL in blue cap):	_____ mL
If applicable, last four digits of residual serum aliquot:		If applicable, last four digits of residual plasma aliquot:	
Time aliquots placed in freezer:	_____[HHMM]	Time aliquots placed in freezer:	_____ [HHMM]
Storage temperature in freezer:	_____ °C	Storage temperature in freezer:	_____ °C
		Buffy coat aliquot created (gray cap, one per 10 mL EDTA tube)	_____ mL

Notes:

Appendix C. CSF Sample and Shipment Notification Form

CSF Sample and Shipment Notification Form

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

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

General Information: **UPS tracking #:** _____

From: _____ Date: _____

Phone: _____ Email: _____

Study: VIVA-MIND **GUID:**_____ **Kit #:** KIT BARCODE

Visit: Screening Week 24 ET

Site ID: _____ **ADCS PTID #:** _____ **Gauge needle used for LP:** 22G 24G

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

CSF Collection:

5. Date of collection:	[MM/DD/YY]	6. Time of collection:	[HHMM]
7. Date subject last ate:	[MM/DD/YY]	8. Time subject last ate:	[HHMM]
9. 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]
2.0ml Sarstedt tube for Roche (2.0 ml expected)	_____ mL
Number of 0.5 mL CSF aliquots created (orange cap):	_____ x 0.5 mL
If applicable, volume of residual CSF aliquot (< 0.5 mL in blue cap):	_____ mL
If applicable, specimen number of residual serum aliquot (last four digits):	
Time frozen:	_____ [HHMM]
Storage temperature in 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: _____