WRAP Protocol Update: V04.2025

Section	Change
	Updated logos and color scheme throughout
3.1	Updated NCRAD contact information - Removed Stephanie Steidel, added Ronae Williams
3.3	Winter Break holiday added
4.2.2	Currently collections are being performed at Milwaukee according to the Madison research sample protocol. Milwaukee clinical lab collections are anticipated to resume in 2024.
6.2	Updated WRAP ID number to remove kit number.
6.2	New label examples added

Wisconsin Registry for Alzheimer's Prevention

UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH
Wisconsin Registry for Alzheimer's Prevention Study (WRAP)

in collaboration with the

National Centralized Repository for Alzheimer's Disease and Related Dementias

Biospecimen Collection, Processing, and Shipment Manual of Procedures

Version 05.2024

Table of Contents

1.0 Abbreviations

AD	Alzheimer's Disease
CITI	Collaborative IRB Training Initiative
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
hs-CRP	High-Sensitivity C-Reactive Protein
IATA	International Air Transport Association
LP	Lumbar Puncture
NACC	National Alzheimer's Coordinating Center
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PST	Plasma Separation Tube
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
SQL DB	SQL Database
SST	Serum Separator Tube
WBC	White Blood Cells

2.0 Purpose

The collection of biofluids is an important part of WRAP. The purpose of this manual is to provide Wisconsin Registry for Alzheimer's Prevention Study (WRAP) 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 WRAP study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

Sites will collect and send the following samples to NCRAD:

- Plasma
- Buffy Coat (DNA Extraction)
- Serum
- 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 WRAP protocol.

3.0 NCRAD Information

3.1 NCRAD Contacts

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

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

Ronae Williams, MSW,BA Clinical Research Coordinator
Phone: 317-278-9082
Email: rdw2@iu.edu

General NCRAD Contact Information
Phone: 1-800-526-2839 or 317-278-8413
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: www.ncrad.org

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

3.2 NCRAD Hours of Operation

Indiana University business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Frozen samples must be shipped **Monday-Wednesday only**.

For packing and shipment details of frozen samples, please refer to Section 8.0 of this protocol.

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

3.3 NCRAD Holiday Observations

Date	Holiday
January 1	New Year's Day
3rd Monday in January	Martin Luther King, Jr Day
4th Monday in May	Memorial Day
June 19	Juneteenth
July 4	Independence Day
1st Monday in September	Labor Day
4th Thursday in November	Thanksgiving
4th Friday in November	Friday after Thanksgiving
December 25	Christmas Day
December 26-31	NCRAD Winter Break

Please note that between December 24th and January 2nd, Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2nd. If at all possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University after the second week in December. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 20th by e-mailing alzstudy@iu.edu, so that they can arrange to have staff available to process incoming samples. **Please see: https://ncrad.org/contact/holiday-closures for additional information.**

- Please note that courier services may observe a different set of holidays.
- Please be sure to verify shipping dates with your courier prior to any holiday.
- **Weekend/holiday delivery must be arranged in advance with NCRAD staff.**

3.4 WRAP Contacts

Sterling Johnson, PhD, Principal Investigator
Phone: (608) 262-9549
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Amy Hawley, Johnson Lab Administrator
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Rachel Weinberg, WRAP Program Manager
Phone: (608) 263-2854
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Beckie Jeffers, Biospecimen Lab Manager
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Celena Ramsey, Primary Milwaukee Site Contact
Phone: (414) 219-7911
Email: celena.ramsey@wisc.edu

Nia Norris, Secondary Milwaukee Site Contact
Phone: (414) 219-5159
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Jo Kaseno, LaCrosse Site Contact
Phone: (608) 392-7187
Email: kaseno.jodi@mayo.edu

4.0 WRAP Laboratory Collection

4.1 Site Required Equipment

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

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

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 frozen specimens to NCRAD, you must provide:

- Dry ice (approximately 45 pounds per shipment)

4.2 Biospecimens Collected at each Visit

4.2.1 Biospecimens Collected at Madison

Whole blood will be collected into up to seven different collection tubes:

2 Red-Top Serum Separation Tubes (One 9 ml and one 4 ml)
4 Purple-Top EDTA Tubes
1 Mint LiHep tube

A Red-Top Serum Separation Tube (9 ml) is processed locally into serum fractions, aliquoted, and frozen at the study site. A portion of these aliquots are then shipped to NCRAD.

Samples for Clinical Labs are collected into one 4 ml Red-Top Serum Separation Tube and one 4 ml Mint LiHep tube after determining a participant's fasting level. These are analyzed and stay on site.

3 Purple-Top EDTA Tubes are processed locally into plasma and buffy coat fractions, aliquoted, and frozen at the study site. A portion of these aliquots are then shipped to NCRAD.

Whole blood from 1 Purple-Top EDTA Tube is aliquoted and frozen at the study site.

CSF is collected only at the Madison site and aliquoted locally. The samples will be frozen at the study site and a portion of these aliquots are then shipped to NCRAD.

4.2.2 Biospecimens Collected at Milwaukee*

Whole blood will be collected into up to eight different collection tubes:

1 Red-Top Serum Separation Tube (9 ml)
3 Gold-Top Tubes
4 Purple-Top EDTA Tubes

The Red-Top Serum Separation Tube is processed locally into serum fractions, aliquoted, and frozen at the study site. These frozen samples are transferred to Madison; a portion of these aliquots are then shipped to NCRAD from Madison.

Samples for Clinical Labs are collected into 3 Gold-Top Tubes after determining a participant's fasting level. These are analyzed and stay on site.

3 Purple-Top EDTA Tubes are processed locally into plasma and buffy coat fractions, aliquoted, and frozen at the study site. These frozen samples are transferred to the Madison site; a portion of these aliquots are then shipped to NCRAD from Madison.

Whole blood from 1 Purple-Top EDTA Tube is aliquoted and frozen at the study site.

CSF is not collected at the Milwaukee site.

*Currently collections are being performed at Milwaukee according to the Madison research sample protocol. Milwaukee clinical lab collections are anticipated to resume in 2024.

4.2.3 Biospecimens Collected at LaCrosse

Whole blood will be collected into up to eleven different collection tubes:

3 Red-Top Serum Separation Tubes (one 9 ml and two 5 ml)
2 Yellow-Top Tubes
4 Purple-Top EDTA Tubes
1 Mint-Top LiHep Tube
1 Gray Top Tube

One 9 ml Red-Top Serum Separation Tube is processed locally into serum fractions, aliquoted, and frozen at the study site. A portion of these aliquots are then shipped to NCRAD.

Samples for Clinical Labs are collected into two 5 ml Red-Top, two Yellow-Top, one LiHep, and one Gray top tube after determining a participant's fasting level. These are analyzed and stay on site.

3 Purple-Top EDTA Tubes are processed locally into plasma and buffy coat fractions, aliquoted, and frozen at the study site. A portion of these aliquots are then shipped to NCRAD.

Whole blood from 1 Purple-Top EDTA Tube is aliquoted and frozen at the study site.

CSF is not collected at the LaCrosse site.

4.3 Informed Consent

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.

Study participants have the option of opting out of biological sample sharing via the consent form. If a participant opts out of sharing biological samples with NCRAD, we will not ship frozen samples.

Biospecimen Collection Table

Biospecimen	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9
Plasma	X	X	X	X	X	X	X	X	X
Buffy Coat (DNA)	X	X	X	X	X	X	X	X	X
Whole Blood	X	X	X	X	X	X	X	X	X
Serum	X	X	X	X	X	X	X	X	X
CSF	Madison only								

4.4 Biospecimens Sent to NCRAD

Frozen samples are to be submitted according to the shipping methods outlined in Section 8.1. Guidelines for the processing, storage location, and timing of sample collection are listed by site in the tables below.

If a sample is not obtained at a particular visit, it should be recorded in the notes section of the **Blood Sample and Shipment Notification Form** (see Appendix A) or **CSF Sample and Shipment Notification Form** (see Appendix B). Submit a copy to NCRAD with a reason provided for the omission and track it as a protocol deviation.

4.4.1 Blood Collection in Madison

Collection Tube	Drawn At	Specimen Type	Aliquot Volume	Total Number of Aliquots	# of Samples sent to NCRAD	Shipping Temperature
1 Serum (Red-Top) Blood Collection Tube (9 ml)	All Visits	Serum	0.5 ml serum aliquots	8	4	Frozen
1 Serum (Red-Top) Blood Collection Tube (4 ml)	All Visits	Clinical Labs	N/A	0	0	N/A
1 Lithium Heparin Blood Collection Tube (4 ml)	All Visits	Clinical Labs	N/A	0	0	N/A
3 EDTA (Purple-Top) Blood Collection Tube (10 ml)	All Visits	Plasma	0.5 ml plasma aliquots	24	4	Frozen
	All Visits	Buffy Coat	~1.0 ml buffy coat aliquots	2	2	Frozen
1 EDTA (Purple-Top) Blood Collection Tube (10 ml)	All Visits	Whole Blood	1 ml whole blood aliquots	10	0	Frozen

4.4.2 Blood Collection in Milwaukee

Collection Tube	Drawn At	Specimen Type	Aliquot Volume	Total Number of Aliquots	# of Samples sent to NCRAD	Shipping Temperature
1 Serum (Red-Top) Blood Collection Tube (9 ml)	All Visits	Serum	0.5 ml serum aliquots	8	4	Frozen
3 (Gold-Top) Blood Collection Tube (5 ml)	All Visits	Clinical Labs	N/A	0	0	N/A
3 EDTA (Purple-Top) Blood Collection Tube (10 ml)	All Visits	Plasma	0.5 ml plasma aliquots	24	4	Frozen
	All Visits	Buffy Coat	~1.0 ml buffy coat aliquots	2	2	Frozen
1 EDTA (Purple-Top) Blood Collection Tube (10 ml)	All Visits	Whole Blood	1 ml whole blood aliquots	10	0	N/A

4.4.3 Blood Collection in LaCrosse

Collection Tube	Drawn At	Specimen Type	Aliquot Volume	Total Number of Aliquots	# of Samples sent to NCRAD	Shipping Temperature
2 Serum (Red-Top) Blood Collection Tube (5 ml)	All Visits	Clinical Labs	3 ml serum aliquot	1	0	Frozen
1 Serum (Red-Top) Blood Collection Tube (9 ml)	All Visits	Serum	0.5 ml serum aliquots	8	4	Frozen
1 Lithium Heparin Blood Collection Tube (3 ml)	All Visits	Clinical Labs	N/A	0	0	N/A
1 Gray Top Blood Collection Tube (1.5ml)	All Visits	Clinical Labs	N/A	0	0	N/A
2 (Yellow-Top) Blood Collection Tube (5 ml)	All Visits	Clinical Labs	N/A	0	0	N/A
3 EDTA (Purple-Top) Blood Collection Tube (10 ml)	All Visits	Plasma	0.5 ml plasma aliquots	24	4	Frozen
	All Visits	Buffy Coat	~1.0 ml buffy coat aliquots	2	2	Frozen
1 EDTA (Purple-Top) Blood Collection Tube (10 ml)	All Visits	Whole Blood	1 ml whole blood aliquots	10	0	Frozen

4.4.4 Cerebrospinal Fluid Collection (Madison site only)

Collection Tube	Drawn At	Specimen Type	Aliquot Volume	Total Number of Aliquots	Number of Samples sent to NCRAD	Shipping Temperature
30 ml screw top centrifuge tubes	All Visits	CSF	0.5 ml CSF aliquots	40	10	Frozen
	All Visits		1 ml CSF aliquot (for local lab)	N/A	N/A	N/A

4.5 Biospecimen Priority for Incomplete Draws

There are times when, during a research visit, the full amount of blood and/or CSF is not collected. In those cases, WRAP will follow the following priority guidelines:

4.5.1 Serum Priorities

Priority #1: NCRAD
Priority #2: WRAP
**If less than 4 aliquots are available, half will stay with WRAP and half will go to NCRAD

4.5.2 EDTA Plasma Priorities

Priority #1: NCRAD Plasma and Buffy Coat
Priority #2: WRAP Plasma
Priority #3: WRAP Whole Blood
**If less than 4 aliquots are available, half will stay with WRAP and half will go to NCRAD

4.5.3 CSF Priorities

Priority #1: 10 Tubes to WRAP
Priority #2: NCRAD

5.0 Specimen Collection Kits, Shipping Kits, and Supplies

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

5.1 NCRAD 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.*

WRAP Blood Kit

Quantity	WRAP Blood Kit Components
4	Cryovial (2 ml) with purple cap
4	Cryovial (2 ml) with red cap
2	Cryovial (2 ml) with clear cap
10	Pre-printed Collection Tube and Aliquot Labels
3	Pre-printed Kit Number Labels
1	Labels for handwritten WRAP ID
1	Cryovial box (holds up to 25 cryovials)

WRAP CSF Kits

Quantity	WRAP CSF Kit Components
10	Pre-printed CSF Aliquot Labels
3	Pre-printed Kit Number Labels
3	Labels for handwritten WRAP ID
1	Cryovial box (holds up to 25 cryovials)

WRAP Supplemental Supply Kit

Quantity	WRAP Blood Supplemental Supply Kit Components
4	4x5 Resealable Bag
20	Labels for handwritten WRAP ID
10	Cryovial (2 ml) with purple cap
10	Cryovial (2 ml) with red cap
10	Cryovial (2 ml) with clear cap
5	Cryovial box (holds up to 25 cryovials)

NCRAD Frozen Shipping Supply Kit

Quantity	NCRAD Frozen Shipping Kit Components
8	Small plastic Biohazard bags with absorbent sheet
1	UPS return airbill pouch
1	Large Shipping box/Styrofoam container
1	Warning label packet (UN3373 label, Dry Ice label, Fragile label)

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

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module.
By Request	Cryovial box (holds up to 25 cryovials)
By Request	Cryovial (2 ml) with purple cap
By Request	Cryovial (2 ml) with red cap
By Request	Cryovial (2 ml) with clear cap
By Request	UPS return airbill
By Request	UPS Clinical Pack
By Request	Shipping container for dry ice shipment (Large Styrofoam box)
By Request	Plastic biohazard bag with absorbent sheet (small)
By Request	Sodium Heparin (Green-Top) Blood Collection Tube (10ml)
By Request	Warning label packet (UN3373 label, Dry Ice label, Fragile label)
By Request	UN3373 label
By Request	Biohazard label
By Request	Dry ice shipping label
By Request	Fine Point Permanent Markers
By Request	Labels for handwritten WRAP ID

5.2 Kit Supply to Study Sites

Each site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits. Please go to: http://kits.iu.edu/WRAP 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 **THREE weeks** for kit orders to be processed and delivered.

6.0 Blood Collection and Processing Procedures

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

In order to ensure the highest quality samples are collected, processed, and stored, it is essential to follow the specific collection, processing, and shipment procedures detailed in the following pages. Collection of blood should be performed after a 12-hour fast. 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.

6.1 Blood Draw Order

6.1.1 Blood Draw Order in Madison:

1. *Tube Use for Clinical Labs:

If able to confirm subject fasting for 12 hours:

- One 4 ml Red-Top Serum Separation Tube for Insulin, B12, and Vitamin D 25-Hydroxy
- One 4 ml PST (Mint Top LiHep) for Lipid Panel, hs-CRP, and Glucose

If unable to confirm subject fasting for 12 hours:

- One 4 ml Red-Top Serum Separation Tube for B12 and Vitamin D 25-Hydroxy
- One 4 ml PST (Mint Top LiHep) for Lipid Panel and hs-CRP

2. Research Kit: One 9 ml Red-Top Serum Separation Tube
   Four 10 ml Purple-Top EDTA Tubes

6.1.2 Blood Draw Order in Milwaukee:

1. Research Kit: One 9 ml Red-Top Serum Separation Tube
   Four 10 ml Purple-Top EDTA Tubes

6.1.3 Blood Draw Order in LaCrosse:

1. Research Kit:
   One 9 ml Red-Top Serum Separation Tube
   Four 10 ml Purple-Top EDTA Tubes

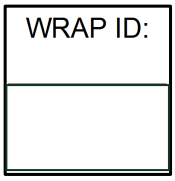
6.2 Labeling Samples for NCRAD

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

6.2.1 Label Type Summary

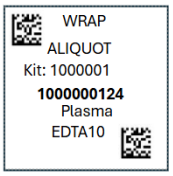
1. Kit Number Label
2. WRAP ID Label
3. Collection and Aliquot Label

Kit Number Labels tie together all specimens collected from one subject at one visit. They should be placed in the designated location on the Blood Sample and Shipment Notification Form and CSF Sample and Shipment Notification Form (if drawn). Also, place one Kit Number Label on the corresponding patient's cryovial box.



WRAP ID Labels are used to document the individual's unique WRAP IDs. These can be used as a backup if Kit Number or Aliquot labels are unavailable. WRAP Staff will write-in the WRAP ID and include this label in the Research Kit provided for blood collection. Per protocol, nurses apply labels to blood collection tubes after verifying participant and while participant is in the room.

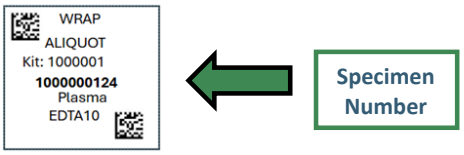
Commented [WRD1]: Change the label image

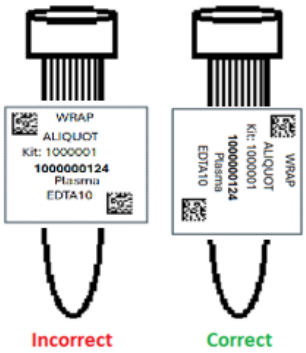


Place one **Collection Tube Label** on Collection Tube and place one **Aliquot Label** on each Aliquot cryovial.

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

- Place Collection Tube and Aliquot Labels on **ALL** NCRAD cryovials **BEFORE** sample collection. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Place cryovials in 25-slot cryobox 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 (see depiction below).
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see attached labeling diagram).
- Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label.

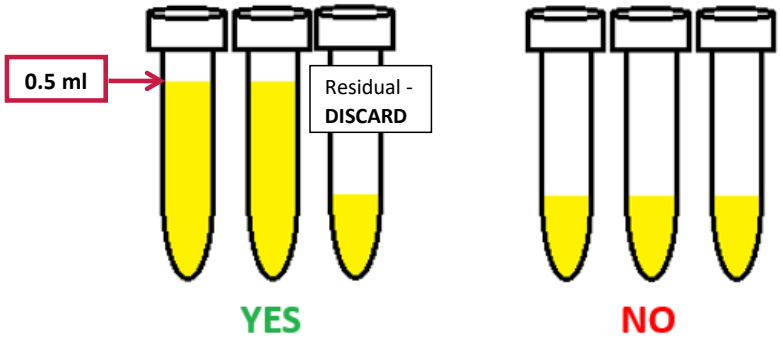




6.3 Filling Aliquot Tubes (Plasma, Serum, and CSF)

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 per the WRAP samples guidelines. If there is any residual volume it is discarded. Ensure that the maximum amount has been aliquoted in as many cryovials as possible after processing the sample. For example, if 1.3 ml of sample is obtained, you should fill 2 cryovials each with 0.5 ml, and discard the remaining 0.3 ml.



Please Note: It is critical for the integrity of future studies using these samples that study staff does not submit residual aliquot tubes (anything under 0.5 ml) to NCRAD. Discard these residual aliquots at your site.

To assist in the preparation and aliquoting of samples, colored caps are used for the NCRAD cryovials. The chart below summarizes the association between cap color and type of cryovial for samples that will be shipped to NCRAD and kept at the site.

NCRAD Cap Color	Sample Type	WRAP Cap Color
Purple Cap	Plasma	Clear Cap
Clear Cap	Buffy Coat	None
Red Cap	Serum	Red Cap
Orange Cap	CSF	Sarstedt Tube

6.4 Whole Blood Collection with 9 ml, 6 ml, and/or 4 ml Serum (Red-Top) Tubes for Serum Collection

1. Set centrifuge to 4°C to pre-chill before use. Please note that the centrifuge could take 30 minutes to chill completely.

2. Label all Serum Collection Tubes based on site standards. All cryovials will be labeled by WRAP Staff prior to arrival in the lab. Place pre-printed NCRAD **SERUM** specimen labels on the four 2 ml cryovials with Red caps. Label the other four 0.5 ml Sarstedt tubes with red caps with WRAP **Serum** labels.

3. Using a blood collection set and a holder, collect blood into **Plain Red-Top Serum Blood Collection Tubes (9 ml, 6 ml, and/or 4 ml)** using your institution's recommended procedure for standard venipuncture technique.

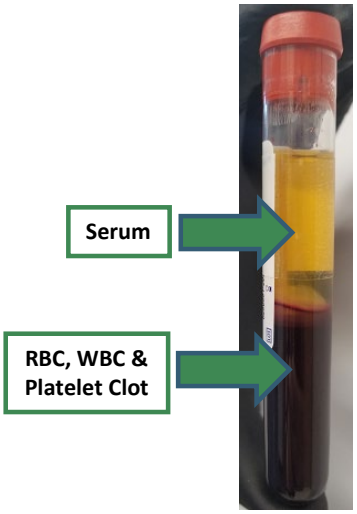
   The following techniques shall be used to prevent possible backflow:
   a. Place participant's arm in a downward position.
   b. Hold tube in a vertical position, below the participant's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube 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 9 ml of blood into the 9 ml tube, 6 ml of blood into the 6 ml tube, and 4 ml of blood into the 4 ml tube.
   a. If complications arise during the blood draw, please note the difficulties on the Blood 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. Allow blood to **clot at room temperature** by placing it upright in a vertical position in a tube rack for at least, but no more than, **30 minutes**.

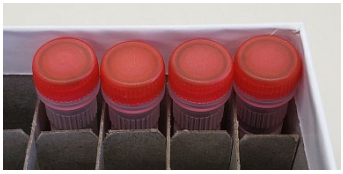
6. After 30 minutes of clotting, centrifuge the collection tube for 10 minutes at 2000 x g at 4°C. **It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum separation (see worksheet in Appendix C to calculate equivalent RPM for spin at 2000 x g).**
    a. While centrifuging, remember to record all times, temperatures and spin rates on the Blood Sample and Shipment Notification Form Appendix A.
    a. **Serum samples need to be spun, aliquoted, and placed in the freezer within 1 hour from the time of collection.**
    b. Record time aliquoted on the Blood Sample Shipment and Notification Form.

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



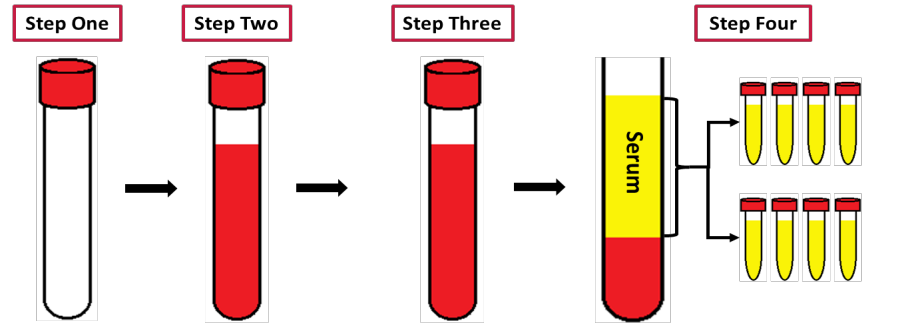
8. Transfer serum into the pre-labeled cryovials with Red caps for NCRAD. The 9 ml serum tube should yield, on average, 4-5 ml of serum, the 6 ml serum tube should yield approximately 3 ml of serum, and the 4 ml serum tube should yield approximately 2 ml of serum. Aliquot 0.5 ml into each red-capped cryovial. Once the cryovials for NCRAD have been filled, aliquot the remaining serum into the cryovials that will remain at the site. Be sure to only place **serum** in cryovials labeled with the **SERUM** labels and red caps.

9. Place the four, labeled cryovials for NCRAD in the 25 cell cryovial box. Place the remaining cryovials in the WRAP serum box. Transfer to **-80°C Freezer within 1 hour of collection**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Blood Sample and Shipment Notification Form.



NCRAD Serum Aliquots

Serum Preparation
(9 ml, 6ml, and 4 ml Red-Top Tubes)



- Store tubes at room temp.
- Each tube should be labeled according to site-specific protocols.

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

- Allow blood to clot for 30 minutes.
- Centrifuge samples at 2000 x g for 10 minutes at 4°C.

- Label four red-capped 2 ml cryovials with preprinted NCRAD "Serum" labels.
- Label four red-capped 0.5 ml cryovials with WRAP "Serum" labels.
- Aliquot 0.5 ml into each cryovial.
- Store serum aliquots upright at -80°C until shipment.
- Spin, aliquot, and freeze aliquots within 1 hour of collection.

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

1. Label all EDTA (Purple-Top) Tubes based on site specific standards. All cryovials will be labeled prior to arrival in the lab. Place pre-printed NCRAD **PLASMA** Aliquot Labels on the four 2 ml cryovials with Purple caps. Place pre-printed NCRAD **BUFFY COAT** specimen label on the two 2 ml cryovials with clear caps. **Place the WRAP EDTA Plasma** labels on the twenty 0.5 ml Sarstedt tubes with clear caps.

2. Please ensure that aliquots are kept in numerical order (by specimen number) throughout the aliquoting and shipping process, from left to right.

3. Using a blood collection set and a holder, collect blood into the **EDTA (Purple-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 participant's arm in a downward position.
   b. Hold tube in a vertical position, below the participant's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

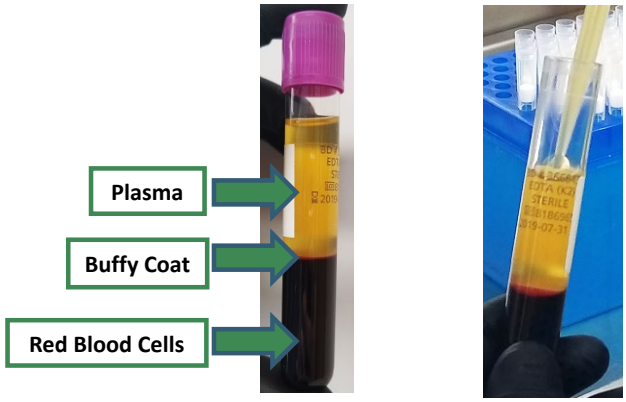
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
   a. If complications arise during the blood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

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

6. As soon as possible, centrifuge balanced tubes for 10 minutes at 2000 x g at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix C to calculate equivalent RPM for spin at 2000 x g).**

a. While centrifuging, remember to record all times, temperatures and spin rates on the Blood Sample and Shipment Notification Form.
b. Record time aliquoted on the Blood Sample Shipment and Notification Form.
c. **Plasma samples need to be spun, aliquoted, and placed in the freezer within <u>1 hour</u> from the time of collection.**

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

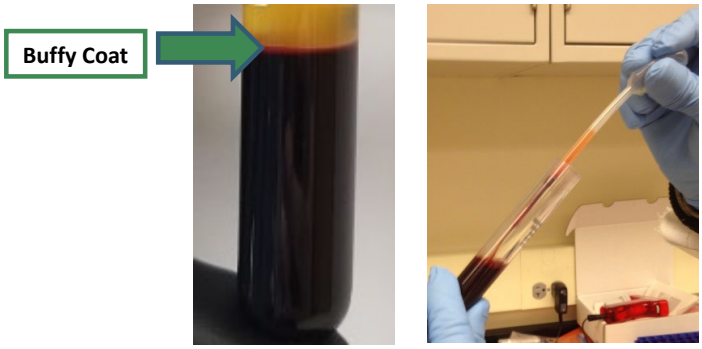


NOTE: When pipetting plasma from the plasma tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

8. Transfer plasma into the pre-labeled cryovials with Purple caps. Each EDTA tube should yield, on average, 4-5 ml of plasma. Aliquot 0.5 ml into each purple-capped cryovial. Be sure to only place **plasma** in cryovials labeled with the **PLASMA** label and Purple caps. Once the purple-capped cryovials for NCRAD have been filled, aliquot the remaining plasma into the twenty clear-capped cryovials and **WRAP EDTA Plasma** labels. Discard any residual plasma below 0.5 ml.

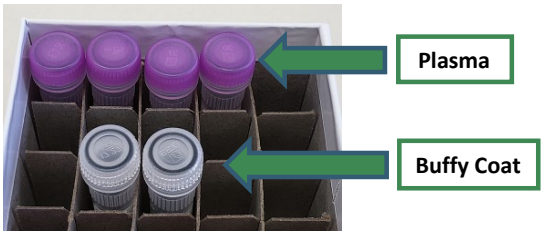
9. Place the labeled, purple-capped cryovials for NCRAD in the 25-slot cryovial box. Place the labeled, clear-capped cryovials with white discs in the WRAP plasma box.

10. After plasma has been aliquoted, pipet the buffy coat layer into the labeled, clear-capped cryovials using a clean pipette. All of the buffy coat should be collected and placed into two cryovials. The buffy coat aliquots are expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovials with the Clear cap and NCRAD **BUFFY COAT** label.



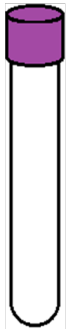
11. Dispose of collection tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.

12. Place the labeled, clear-capped cryovials in the same 25-slot cryovial box with the purple-capped cryovials and transfer to **-80°C Freezer within 1 hour of the time of collection**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice.



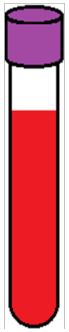
Plasma and Buffy Coat Preparation
EDTA Purple-Top Tube (10 ml)

Step One



- Store tubes at room temp.
- Each tube should be labeled according to site-specific protocols.

Step Two

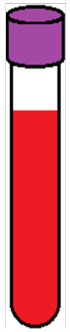


- Collect blood in EDTA Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three

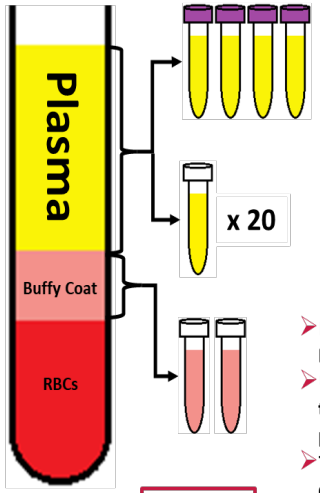
- Immediately after blood draw, invert tube 8-10 times to mix samples.

Step Four



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

Step Five



- Label purple-capped 2 ml cryovials with NCRAD "PLASMA" labels.
- Aliquot 0.5 ml into each cryovial.
- Store plasma aliquots at -80°C until shipment.

- Label clear-capped 0.5 ml cryovials with WRAP "EDTA Plasma" labels.
- Aliquot 0.5 ml into each cryovial.
- Store plasma aliquots at -80°C.

Step Six

- Label clear-capped 2 ml cryovials with NCRAD "BUFFY COAT" labels.
- Using a clean transfer pipette, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat into the cryovials.
- Store buffy coat aliquots at -80°C until shipment.
- Spin, aliquot, and freeze aliquots within 1 hour of collection

7.0 CEREBROSPINAL FLUID COLLECTION

*****Important Note*****
CSF samples should be collected following a minimum 4-hour fast.
Only water is permitted until the lumbar puncture is completed.

7.1 General CSF Guidelines

- 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.
- Participants taking an anti-platelet agent (e.g. aspirin) may, at the discretion of the site clinician, be discontinued from that agent for a period of time prior to lumbar puncture and/or continue off agent for a period of time post LP. Participants who are taking anticoagulants (e.g. warfarin (Coumadin) and/or dabigatran (Pradaxa)) may not undergo an LP and are not suitable to participate in this study.
- 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). Check expiration dates on all supplies, especially lidocaine.

7.2 CSF Collection Method

LPs for CSF collection should be performed using a 24 or 25 gauge Sprotte needle. Sites must designate the method of CSF collection for data tracking purposes. It is recommended that CSF be obtained from participants in a sitting position.

7.3 Scheduling the LP

All LPs should be performed in the morning if possible. Availability of staff and facilities for next day blood patch should be considered when scheduling LPs. CSF amyloid levels can vary depending upon the time of day the sample is collected. It is important for the time of day of collection to remain consistent across study visits. The LP should be rescheduled if the participant does not feel well or is febrile.

7.4 Setting up the LP

1. Position the participant in either the sitting (preferred) or in the lateral decubitus position; aim for maximum flexion of the lumbar spine with the hips, shoulders and head in line.

2. Draw an imaginary line between the top of the iliac crests until it intersects with the spine at L4, then feel for the L4/L5 or L3/L4 interspace; mark this area if necessary.

3. Prepare the skin with chlorhexidine (or povidone-iodine if chlorhexidine allergy) and allow adequate time to dry (1-2min).

4. Open the sterile pack on the bedside table; the nurse will drop sterile 5 mL syringes and caps onto the sterile field.

5. Wash hands with soap and water. Apply sterile gloves.

6. Set up sterile drapes (2), applying one to the back and one on the bed overlapping the 1st drape.

7. Place sterile 4x4 gauze on top of sterile field on the bed.

8. Plunge the 5 mL syringes (6) to break the seals; place 1 syringe on the sterile field on the bed, the other 5 will remain on the sterile field on the bedside table.

9. Inform the coordinator which size spinal needle you will use (i.e. 25 G. Sprotte vs. 24 G. Sprotte); the nurse will drop the spinal needle onto the sterile field. Goal is to use the 25 Gauge as it will reduce risk for bleeding, pain at site, and headache. Try to use the shortest length possible to help with handling.

10. Draw up 5 mL of 1% lidocaine from the glass vial with the filter needle or filter tube attached to a 5 mL syringe (to prevent glass shards from being aspirated into syringe), then remove the filter needle/tube; attach a 25 G. 1 ½ in. needle to inject Lidocaine.

7.5 Performing the LP

1. Print CSF Sample and Shipment Notification Form.

2. Confirm all supplies, including dry ice (~30-45 lbs), are available.

3. Label the forty Sarstedt Tubes with provided **CSF** labels. Ten are labeled with NCRAD CSF Labels, 30 with WRAP CSF Labels. Do **NOT** open and label the 30 ml conical polypropylene tubes that will be kept sterile to collect the CSF.

4. Pre-cool the centrifuge.

5. Anesthetize the skin at the insertion site by holding the needle with bevel up, syringe parallel to the back, and forming a 1-2 cm wheal under the skin. Wait one minute and okay to apply some pressure to the wheel to help it diffuse. Then anesthetize the deeper tissue under the insertion site with the needle perpendicular to the back, injecting 2-4 mL of Lidocaine in multiple directions (superior, inferior, right and left).

6. Position the needle in the midline of the interspace and angle the needle to aim toward the umbilicus.

7. Insert the introducer approximately half way in; start to advance the needle and continue to advance through increased resistance until there is a fall in resistance.

8. Remove the stylet and check for CSF, if no CSF is obtained, replace the stylet and advance the needle slightly and recheck for CSF; continue this process until CSF obtained. Make sure the stylet is completely in with ridge at the base aligned properly.

9. If the needle meets resistance and is unable to be advanced, withdraw the needle into the introducer and withdraw the introducer to redirect the needle and attempt the process again until CSF is obtained.

10. Once CSF is obtained, connect syringe #1 and collect 0.5-1 mL of CSF for waste; if fluid is blood-tinged, more can be collected until fluid is clear; discard this syringe.

11. Connect syringe #2 and collect 1 mL of CSF to send to the UW lab for cell count; hand this syringe to the nurse. Withdraw fluid at a rate so it takes between 2-3 minutes to get all 5mL.

12. Collect the remaining CSF in syringes #3-6, filling the 5 mL syringes, for a total of 20 mL; hand the syringe to the nurse after disconnecting each one.

13. Within 15 minutes of collection, centrifuge samples at 4°C at 2000 x g for 10 minutes **(see worksheet in Appendix C to calculate equivalent RPM for spin at 2000 x g).**

14. After CSF is collected replace the stylet, twist the needle 180 degrees and withdraw it into the introducer, then remove the needle and introducer; hold brief pressure over the site with gauze, clean dried blood with an alcohol wipe, then cover with a round bandage.

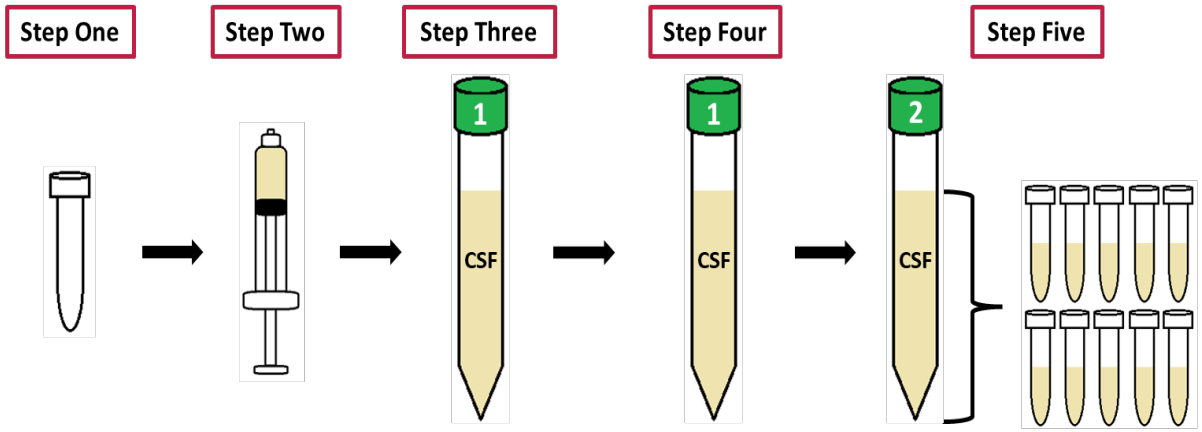
15. Using a clean transfer pipette, transfer all CSF into a new 30 ml conical tube, leaving the pellet in the bottom of the first tube. Gently invert the 30 ml conical tube 3-4 times to mix the sample.

16. Pipet 0.5 ml of supernatant directly into CSF aliquot cryovials (Sarstedt Tubes) for NCRAD. Fill the remaining aliquots with 0.5 ml for local storage.

17. Place the labeled cryovials (Sarstedt tubes) for NCRAD in the 25 cell cryovial box and freeze. Place the other aliquots in Sarstedt Tubes into WRAP CSF box. Transfer samples to **-80°C Freezer within 1 hour of collection time**. Record time and storage temperature of freezer on CSF Sample and Shipment Notification Form. The CSF cryovials may be placed into the same cryovial box as the patient's blood specimens for shipment.

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

CSF Preparation



Step One

- Label cryovials with pre-printed specimen labels prior to collection.

Step Two

- Collect initial 0.5-1 ml CSF for waste (if bloody, collect CSF until cleared of blood). Discard syringe #1.
- Collect 1 ml CSF in syringe #2 and transfer syringe to local lab for cell count.

Step Three

- Collect 20 ml CSF using syringes #3-6 into one 30 ml conical tube.

Step Four

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

Step Five

- Using a clean transfer pipette, transfer all CSF into a new 30 ml conical tube leaving the pellet in the bottom of the first conical tube.
- Gently invert the 30 ml conical tube 3-4 times to mix the sample.
- Aliquot 0.5 ml into the CLEAR-capped cryovials.
- Store CSF aliquots upright at -80°C until shipment.
- Spin, aliquot, and freeze aliquots within 1 hour of collection

8.0 Packaging & Shipping Instructions

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

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

8.1 Frozen Packaging Instructions

*****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

8.2.1 NCRAD Packaging Instructions – Frozen Shipments

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

2. Notify NCRAD of shipment by emailing NCRAD coordinators at alzstudy@iu.edu. Attach the following to the email:
   a. Completed Blood Sample and Shipment Notification Form to the email notification. (See Appendix A and/or Appendix B for the NCRAD sample forms)
   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 all of one subject's frozen labeled aliquots of plasma, buffy coat, serum, and CSF (if applicable) into one 25-slot cryovial box.
   a. A cryovial box should contain all of the specimens from the same patient, per time point.
   b. **Batch shipping should be performed every three months, or when specimens from eight participants accumulate,**

whichever is sooner. WRAP will plan to ship on a weekly basis unless unnecessary.

Plasma, Buffy Coat, Serum, and CSF Samples

Plasma, Buffy Coat, and Serum Samples

4. Label the outside of each cryovial box with the kit number label(s). Please place the cryovial boxes containing blood derivatives (and CSF, if drawn) in one biohazard bag.

5. As the cryovial box is placed in the plastic biohazard bag, do NOT remove the absorbent material found in the bag. Seal according to the instructions on the bag. The kit number label(s) should be placed on each cryovial box prior to inserting into the biohazard bag.

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

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

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

9. The inner Styrofoam shipping container must contain approximately 30-45 lbs of dry ice. The dry ice should entirely fill the inner box and be placed on top of the biohazard bags to ensure the frozen state of the specimens.

Shipping container filled with dry ice

10. 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.

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

12. Apply all provided warning labels and the UPS return airbill to the outside of package, taking care not to overlap labels. Return airbill should go on top of package and should NOT overlap package seams.

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

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

13. If possible, hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off. If storage in a -80°C freezer until UPS pick-up is not

possible, package samples no more than 4 hours before the expected pick-up time.

14. Specimens should be sent to NCRAD via UPS Next Day Air. **Frozen shipments should be sent Monday through Wednesday** to avoid shipping delays on Thursday or Friday.

15. 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.

16. Remember to complete the Sample Form(s) (Appendix A and/or Appendix B), include a copy in your shipment, AND notify the NCRAD Study Coordinator by email at alzstudy@iu.edu (include UPS tracking number in email) IN ADVANCE to confirm the shipment.

SHIP ALL FROZEN SAMPLES MONDAY - WEDNESDAY ONLY!

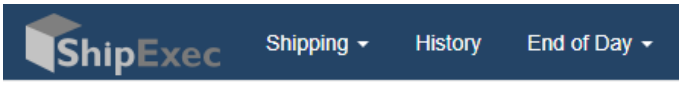
BE AWARE OF HOLIDAYS!!

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

8.3 Frozen Shipping Instructions

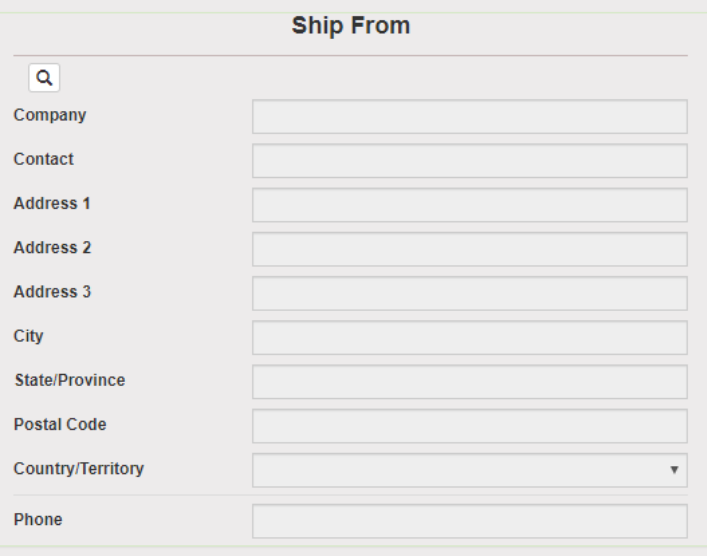
1. Log into the ShipExec Thin Client at kits.iu.edu/UPS.
    a. If a new user or contact needs access, please reach out to your study contact for access.

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



3. Select your study from the “Study Group” drop down on the right side of the main screen. Choosing your study will automatically filter the address book

to only addresses within this study 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.

4. 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.

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

If your site does not already have a daily UPS pickup, you can schedule one here.

c. Click the blue Pickup Request button. Enter the earliest pickup time and latest pickup time in 24-hr format.
d. Give a name & phone number of someone who the UPS driver can call if having issues finding the package
e. 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.

6. 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.

7. Fold airbill and place inside plastic UPS sleeve.

8. Peel the back off of the UPS sleeve and stick the sleeve to the package.

9.0 Data Queries and Reconciliation

The Sample and Shipment Notification form must be completed on the day that samples are collected because 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.

NCRAD will collaborate with the Madison Sample Manager at Madison site to reconcile information captured in the local database compared to samples received and logged at NCRAD. Information that appears incorrect in the database will be queried through the standard system. Additional discrepancies that may be unrelated to data entry will be resolved with the Principal Investigator in a separate follow up communication. If applicable, a non-conformance report will be provided to sites on a monthly basis.

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 Blood Sample and Shipment Notification Form and logged at NCRAD compared to information entered into the NACC 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

10.0 Appendices

Wisconsin Registry for Alzheimer's Prevention
UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH

Appendix A: Blood Sample and Shipment Notification Form

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

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

From:_____ **UPS tracking #:**_____

Phone:_____ **Email:**_____

Site (circle one)**:** Madison Milwaukee La Crosse

WRAP ID:_____

Visit Number: _____

Sex: ☐ M ☐ F **Year of Birth:** _____

Kit #: KIT BARCODE

Blood Collection:

Date of Draw:	_____	Time of Draw:	_____ ☐AM ☐PM
Date subject last ate:	_____	Time subject last ate:	_____ ☐AM ☐PM
CSF Collected?	☐ Yes ☐ No		

Blood Processing:

Serum (Red-top) Tube (9 ml)		**Plasma & Buffy Coat (Purple-top) Tube (10 ml)**	
Original volume drawn:	_____ml	Original volume drawn:	_____ml
Time spin started:	_____ ☐AM ☐PM	Time spin started:	_____ ☐AM ☐PM
Duration of centrifuge:	_____ minutes	Duration of centrifuge:	_____ minutes
Temp of centrifuge:	_____ °C	Temp of centrifuge:	_____ °C
Rate of centrifuge:	_____ x g	Rate of centrifuge:	_____ x g
Time aliquoted:	_____ ☐AM ☐PM	Time aliquoted:	_____ ☐AM ☐PM
# of 0.5 ml serum aliquots **(red-capped cryovials)**:	_____	# of 0.5 ml plasma aliquots **(purple-capped cryovials)**:	_____
		# of buffy coat aliquots **(clear-capped cryovials)**:	_____
Time aliquots placed in freezer:	_____ ☐AM ☐PM	Time aliquots placed in freezer:	_____ ☐AM ☐PM
Storage temperature in freezer:	_____ °C	Storage temperature in freezer:	_____ °C

Notes:
_____

Wisconsin Registry for Alzheimer's Prevention
UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH

Appendix B: 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

From:_____ **UPS tracking #:**_____

Phone:_____ **Email:**_____

Site: Madison

WRAP ID:_____

Sex: ☐ M ☐ F **Year of Birth:** _____

Kit #: KIT BARCODE

CSF Collection:

Date of Draw:	_____	Time of Draw:	_____ ☐AM ☐PM
Date subject last ate:	_____	Time subject last ate:	_____ ☐AM ☐PM
Collection process: ☐ Gravitational **OR** ☐ Pull			

CSF Processing:

Original volume drawn:	_____ml
Time spin started:	_____ ☐AM ☐PM
Duration of centrifuge:	_____ minutes
Temp of Centrifuge:	_____ °C
Rate of centrifuge:	_____ x g
Time aliquoted:	_____ ☐AM ☐PM
Number of 0.5 ml CSF aliquots created **(clear-capped cryovials)**:	_____
Time aliquots placed in freezer:	_____ ☐AM ☐PM
Storage temperature in freezer:	_____ °C

Notes:

_____

_____

Appendix C: Rate of Centrifuge Worksheet

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

Submitter Information

Name: **Site:**

Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

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

Calculating RPM from G-Force:

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

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

Comments:

Please send this form to NCRAD Study Coordinator at alzstudy@iu.edu