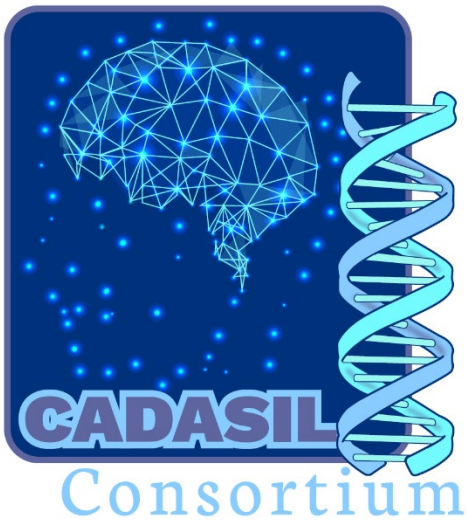
CADASIL Manual of Procedures Update: V1.2

Section	Change
Document Footer	The version date was updated for this amendment.
Title Page	Title page updated to reflect version change.
Table of Contents	Updated page numbers.
Section 3.0	New NCRAD Laboratory Manager
Section 6.0	Updates to reflect new labels.
Section 6.3	Updates to reflect new labels.
Section 6.4	Updates to reflect new labels.
Section 6.5	Updates to reflect new labels.
Section 8.0	Added image of a large frozen shipping container and indicated how much dry ice and how many cryoboxes should be included in one batch shipment.
Throughout Document	1. Updated kit labels to reflect new Collection and Aliquot labels included in kits since NCRAD moved to a new LIMS. 2. Added Alternate Text to all graphics and removed most text boxes for ADA accessibility. 3. Updated NCRAD website links. 4. Updated NCRAD logo and document theme. 5. Removed wording.



Cerebral Autosomal Dominant Arteriopathy with Sub-cortical Infarcts and Leukoencephalopathy Study

in collaboration with the

National Centralized Repository for Alzheimer's Disease and Related Dementias

Biofluid Collection, Processing and Shipment Manual of Procedures

Version 1.2
April 2025

Table of Contents

1.0 Abbreviations

AD	Alzheimer's Disease
BL	Baseline visit
CADASIL	Cerebral Autosomal Dominant Arteriopathy with Sub-cortical Infarcts and Leukoencephalopathy
COINS	Collaborative Informatics and Neuroimaging Suite
DNA	Deoxyribonucleic Acid
EDC	Electronic Data Capture
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
NACC	National Alzheimer's Coordinating Center
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PBMC	Peripheral Blood Mononuclear Cell
PHI	Protected Health Information
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RNA	Ribonucleic Acid
RPM	Revolutions Per Minute
SST	Serum separator tube
TReNDS	Translational Research in Neuroimaging and Data Science
UPS	United Parcel Service

2.0 Purpose

The collection of biofluids is an important part of the Cerebral Autosomal Dominant Arteriopathy with Sub-cortical Infarcts and Leukoencephalopathy Study (CADASIL). The purpose of this manual is to provide study staff (PIs, study coordinators, phlebotomists) at the various study sites with instructions for collection and submission of biological samples for CADASIL study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

The following samples will be sent to NCRAD:

- Plasma
- Buffy Coat (DNA Extraction)
- RNA
- Serum

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

These procedures are relevant to all study personnel responsible for processing specimens being provided to NCRAD for the CADASIL protocol.

3.0 NCRAD Information

3.1 NCRAD Contacts

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

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

Michael Edler, PhD, Laboratory Director
Phone: 317-278-2209
Email: mcedler@iu.edu

Zoë McManus, BA, CCRP, Study Coordinator
Phone: (317) 278-9086
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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
CADASIL Study Specific Webpage: https://ncrad.org/coordinate-studies/cadasil

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

1.2 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: Service Alerts and Shipping Updates.

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: UPS Holiday Schedule | UPS - United States.

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
June 19	Juneteenth (observed)
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
December 26-31	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 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 visit NCRAD - Holiday Closures **for additional information.**

4.0 NCRAD Laboratory Collection

4.1 Site Required Equipment

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

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquet
- Alcohol Prep Pad
- Gauze Pad

- Bandage
- Butterfly needles and hub
- Microcentrifuge tube rack
- Sharps bin and lid
- Wet Ice Bucket
- Wet ice
- Pelleted dry ice

In order to process samples consistently across all projects and ensure the highest quality samples possible, project sites must have access to the following equipment:

- **Centrifuge capable of ≥ 2000 x g at room temperature**
- **-80°C Freezer**

In order to ship specimens, you must provide:

- Pelleted dry ice (about approximately 30-45 lbs. per shipment)

4.2 Biofluid Collection Schedules

CADASIL Blood-Based Biomarker Collection Schedule:

	Visit 1 (Baseline)	Visit 2 (18 month)	Visit 3 (36 month)
Plasma	X	X	X
Buffy Coat	X	X	X
RNA	X	X	X
Serum	X	X	X

Whole blood is collected in three different types of tubes for shipment to NCRAD: (4) 10 mL lavender-top EDTA tubes, (2) 2.5 mL PAXgene™ blood collection tubes, and (1) 8.5 mL tiger-top SST tube.

- The (4) 10 mL EDTA are processed locally into plasma and buffy coat fractions. They are then aliquoted, frozen at the study site, and shipped to NCRAD.
- The (2) 2.5 mL PAXgene™ tubes are frozen locally without further processing and shipped to NCRAD.
- The (1) 8.5 mL SST tubes are processed locally into serum fractions. It is then aliquoted, frozen at the study site, and shipped to NCRAD.

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

Frozen samples are to be submitted according to the shipping methods outlined in Section 8.1 Frozen Packaging Instructions for the processing, storage location, and timing of sample collection are listed in the following table.

Biospecimen Collection Chart

4.2.1 Biospecimen Collection Chart: Baseline, 18-month, and 36-month Visits

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/Aliquoting	Tubes to NCRAD	Ship
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	**EDTA (Lavender-Top) Blood Collection Tube (10 mL)**	**4**	**N/A**	**N/A**	**N/A**
	PLASMA: 0.75 mL cryovials	8	0.25 mL plasma aliquot per 0.75 mL cryovial (Micronic™ purple cap)	8	**Frozen**
	PLASMA: 2.0 mL cryovials	12	1.5 mL plasma aliquot per 2.0 mL cryovial (Micronic™ purple cap)	12	
	PLASMA RESIDUAL: 2.0 mL cryovials	1	1.5 mL plasma aliquot per 2.0 mL cryovial (Micronic™ blue cap)	1	
	BUFFY COAT: 2.0 mL cryovials	4	1 mL buffy coat aliquot per 2.0 mL cryovial (Micronic™ gray cap)	4	
Whole blood for RNA extraction	**PAXgene™ Blood Collection Tube (2.5 mL)**	**2**	**N/A**	**2**	**Frozen**
Whole blood for isolation of serum	**SST (Tiger-Top) Blood Collection Tubes (8.5 mL)**	**1**	**N/A**	**N/A**	**N/A**
	SERUM: 0.75 mL cryovials	8	0.25 mL serum aliquot per 0.75 mL cryovial (Micronic™ red cap)	8	**Frozen**
	SERUM: 2.0 mL cryovials	1	1.5 mL serum aliquot per 2.0 mL cryovial (Micronic™ red cap)	1	
	SERUM RESIDUAL: 2.0 mL cryovials	1	1.5 mL serum aliquot per 2.0 mL cryovial (Micronic™ blue cap)	1	

If a sample is not obtained at a particular visit, it 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 and track it as a protocol deviation.

5.0 Specimen Collection Kits, Shipping Kits, and Supplies

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD, 2) the Blood Supplemental Supply Kit, 3) the Frozen Shipment Kit, 4) clinical lab supplies (with the exception of pelleted dry ice and equipment supplies listed in Section 4.1). The provided materials include blood tubes, pipettes, boxes for serum/plasma/buffy coat aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, Site and CADASIL ID Labels, Collection and Aliquot Tube Labels will all be provided by NCRAD. Details regarding the blood 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 6.1.

5.1 Specimen Collection Kit Contents

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

CADASIL Participant Blood Supply Kits

Quantity	Participant Blood Supply Kit Components
4	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
2	PAXgene™ Blood Collection Tube (2.5 mL)
1	SST (Tiger-Top) Blood Collection Tubes (8.5 mL)
8	0.75 mL Micronics Tube - RED
1	2 mL Micronics Tube - RED
8	0.75 mL Micronics Tube - PURPLE
12	2 mL Micronics Tube - PURPLE
4	2 mL Micronics Tube - GRAY
2	2 mL Micronics Tube - BLUE
6	Disposable pipette (3 mL)
1	Conical tube (50 mL)
19	Cryovial Labels
7	Collection Tube Labels
8	Site and CADASIL ID Labels
6	Kit Number Labels
1	Micronic 48 Rack with high cover (2 mL cryovials)
1	Micronic 96 Rack with high cover (0.75 mL cryovials)
2	Resealable bubble tube pouch (on roll)
1	Resealable small poly bag (4" x 6")
1	Resealable bag

CADASIL Blood Supplemental Supply Kit

Quantity	Blood Supplemental Kit Components
8	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
4	PAXgene™ Blood Collection Tube (2.5 mL)
2	SST (Tiger-Top) Blood Collection Tubes (8.5 mL)

16	0.75 mL Micronics Tube - RED
2	2 mL Micronics Tube - RED
16	0.75 mL Micronics Tube - PURPLE
24	2 mL Micronics Tube - PURPLE
8	2 mL Micronics Tube - GRAY
4	2 mL Micronics Tube - BLUE
12	Disposable pipette (3 mL)
2	Conical tube (50 mL)
14	Site and CADASIL ID Labels
1	Micronic 48 Rack with high cover (2 mL cryovials)
1	Micronic 96 Rack with high cover (0.75 mL cryovials)
12	Resealable small poly bag (4" x 6")
1	Resealable bag

CADASIL Frozen Blood Shipping Supply Kit

Quantity	Frozen Blood Shipping Supply Kit Components
1	Dry Ice label
1	UN3373 sticker
1	Fragile label
1	Airbill Sleeve
1	Shipping box/Styrofoam container for dry ice shipment
1	Resealable bag
4	Large Biohazard shipping bag
4	250 mL absorbent sheet

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module.
By Request	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
By Request	PAXgene™ Blood Collection Tube (2.5 mL)
By Request	SST (Tiger-Top) Blood Collection Tubes (8.5 mL)
By Request	0.75 mL Micronics Tube - RED
By Request	2 mL Micronics Tube - RED
By Request	0.75 mL Micronics Tube - PURPLE
By Request	2 mL Micronics Tube - PURPLE
By Request	2 mL Micronics Tube - GRAY
By Request	2 mL Micronics Tube - BLUE
By Request	Disposable pipette (3 mL)
By Request	Conical tube (50 mL)
By Request	Site ID Label
By Request	Micronic 48 Rack with high cover (2 mL cryovials)
By Request	Micronic 96 Rack with high cover (0.75 mL cryovials)
By Request	Dry Ice label
By Request	UN3373 sticker
By Request	Fragile label
By Request	Airbill Sleeve
By Request	Shipping box/Styrofoam container for dry ice shipment

By Request	Resealable bubble tube pouch
By Request	Large Biohazard shipping bag
By Request	250 mL absorbent sheet

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/cadasil 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.
Due to ongoing supply limitations, we ask that you please only order as many kits and extra supplies that you will be able to use in the next 30 days. Doing so allows us to fulfill as many kit requests as possible without depleting stock for other kit requests in our queue. If we are not able to fulfill any part of your request due to supplies being out of stock, we will reach out about those individually

6.0 Blood Collection and Processing Procedures

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

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 biomarkers should be collected after a minimum 6-hour fast, preferably in the morning. Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood. Please note that the centrifuge may take 30 minutes to cool, so please plan accordingly.

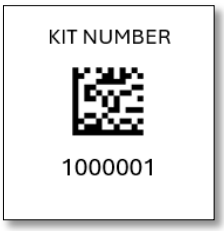
Draw blood in the following order:

1. **EDTA (Lavender-Top) Blood Collection Tube (10 mL) for Plasma and DNA x 4**
2. **PAXgene™ Blood Collection Tube (2.5 mL) for RNA x 2**
3. **SST (Tiger-Top) Blood Collection Tubes (8.5 mL) for Serum x 1**

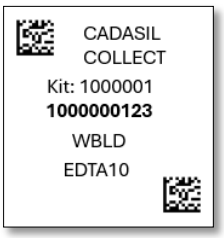
6.1 Labeling Samples

Label Type Summary:

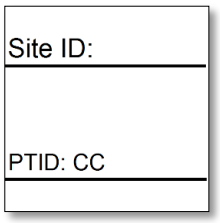
1. Kit Number Label
2. Collection Tube Label
3. Site and CADASIL ID Label
4. Cryovial Tube Label



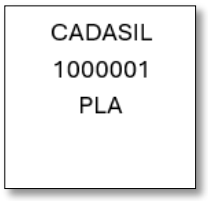
The **Kit Number Labels** do not indicate a specimen type but are affixed on the Biological Sample and Shipment Notification Forms and on specific packing materials.



The **Collection Tube Labels** for blood derivatives are placed on all collection tubes.

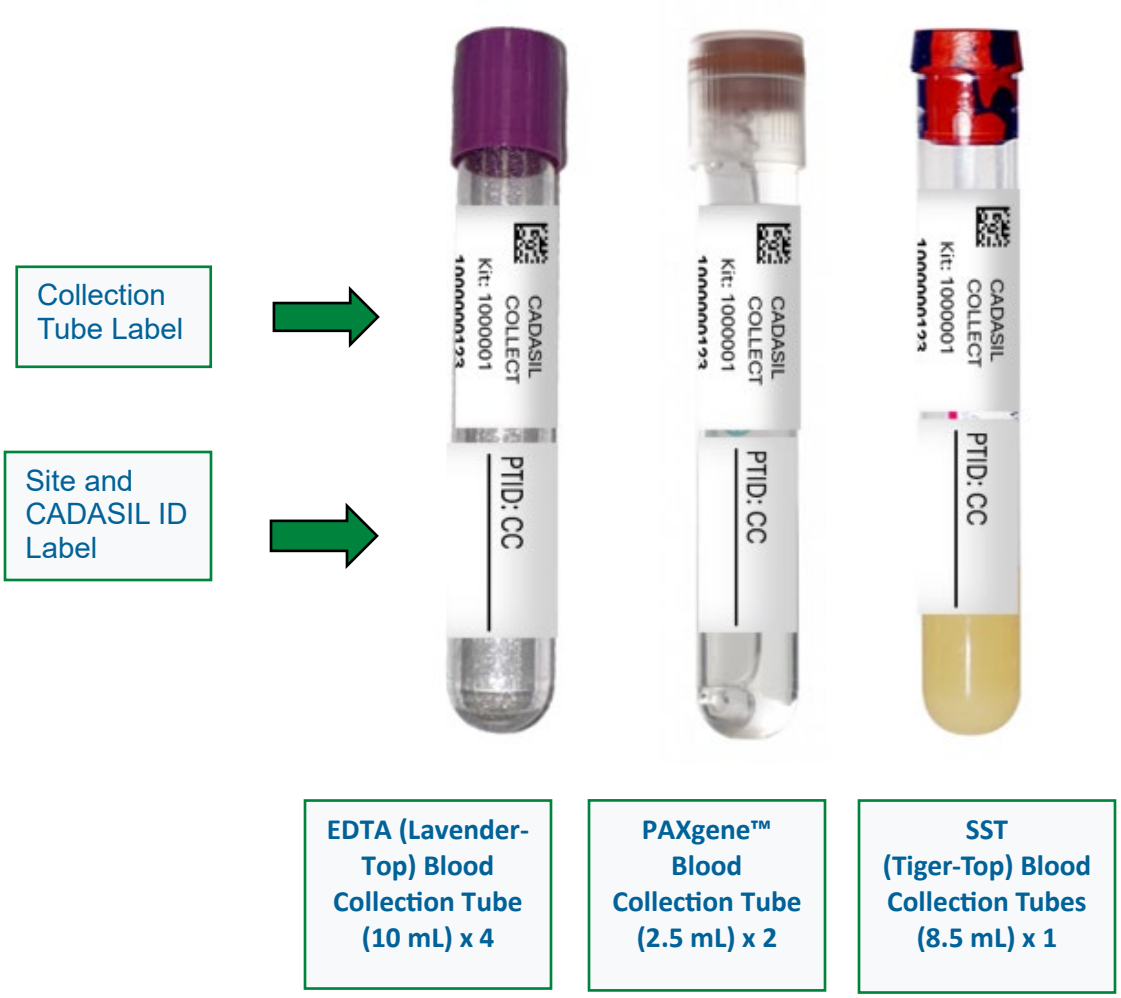


The **Site and CADASIL ID Labels** are placed on all collection tubes.



The **Cryovial Tube Labels** for blood derivatives are placed on all 2.0 mL aliquot tubes. The 0.75 mL cryovials will be pre-etched with the sample type.

Important Note
Each collection tube will contain two labels: the Collection Tube Label and the Site and CADASIL 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 CADASIL ID label.

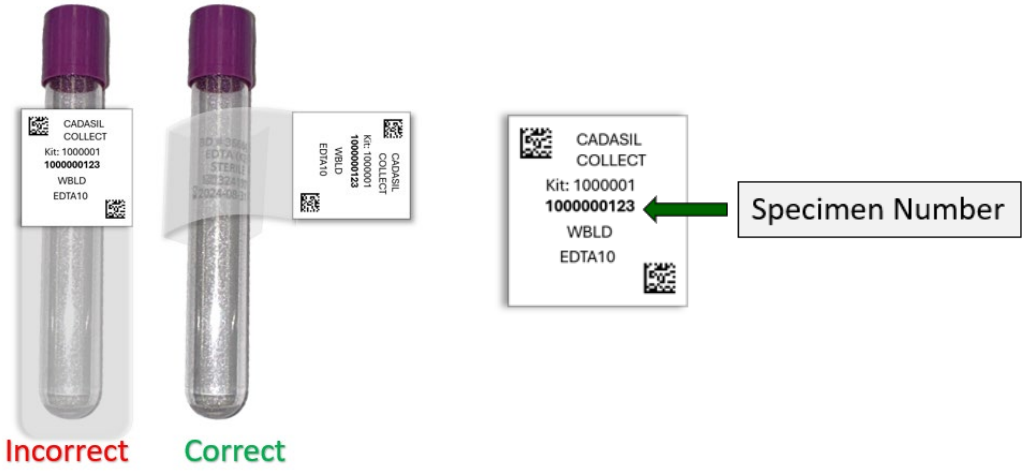


In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place blood collection tube labels on **ALL** collection tubes and cryovial tube labels on **ALL** 2.0 mL cryovials **BEFORE** sample collection, sample processing, or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.

- Using a fine point permanent marker, fill-in and place the Site and CADASIL ID Labels on the collection tubes only (EDTA, PAXgene™, SST) **BEFORE** sample collection, processing, or freezing. These labels are placed on the collection tubes in addition to the Collection Tube Labels.

- Collection Tube Labels contain 2D barcodes on the top left-hand and bottom right-hand side of the label. Place label horizontally on the tube with either the left or right edge of the label facing towards the tube cap.



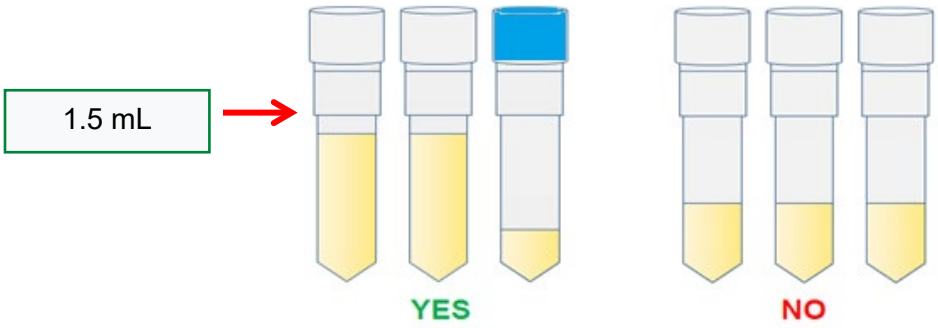
- Place the cryovial tube labels on the 2.0 mL aliquot tubes in the same manner as the collection tube labels. Make sure to **NOT** cover the barcode etched on the cryovials. (see diagram below).

- Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label.
If there are any unused cryovials, please do not send the empty cryovials to NCRAD. These unused cryovials (ensure labels are removed) can be saved as part of a supplemental supply at your site or the cryovials can be disposed of per your site's requirements.

6.2 Filling Aliquot Tubes (Plasma and Serum)

In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each cryovial should be filled to the assigned volume with the respective biological

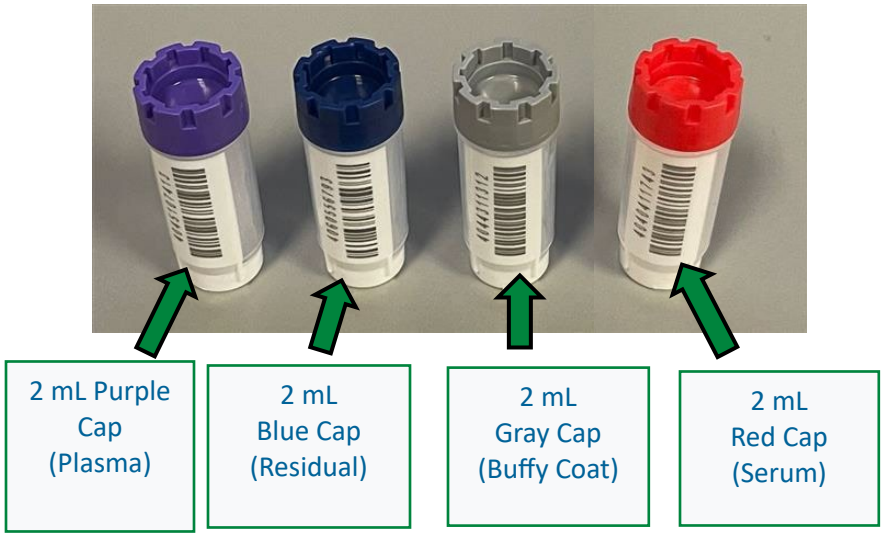
material after processing is completed (refer to detailed processing instructions for average yield per sample).
Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample.
Aliquot the remaining biologic material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 3.6 mL of sample is obtained, you should fill 2 cryovials each with 1.5 mL, and one additional cryovial with the remaining 0.6 mL.

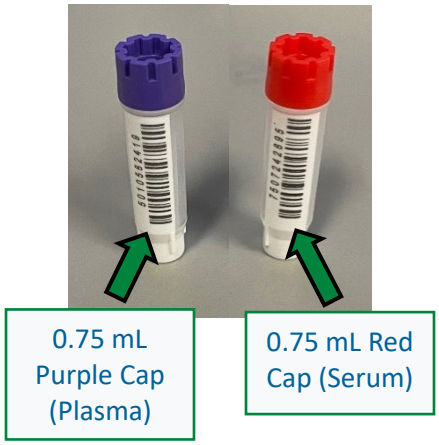


Please note: It is critical for the integrity of the samples that study staff note if a 2.0 mL cryovial contains a residual volume (anything under 1.5 mL). Please highlight that the aliquot contains a small volume by utilizing the blue cryovial cap provided in each kit. Please record the specimen number and volume of the residual aliquot on the Biological Sample and Notification Form.

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

Cap Color	Sample Type
Purple Cap (0.75 mL and 2.0 mL)	Plasma
Blue Cap (2.0 mL)	Residual (plasma and serum)
Gray Cap (2.0 mL)	Buffy Coat
Red Cap (0.75 mL and 2.0 mL)	Serum





6.3 EDTA (Lavender-Top) Blood Collection Tube (10 mL) for Plasma and Buffy Coat x 4

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 aliquots)

> **Important Note:** Ensure all tubes are not expired prior to collection and processing of samples.

1. Place completed Site and CADASIL ID Labels and pre-printed "**WBLD EDTA10**" Collection Tube Labels (with "COLLECT" under the study name) on the 10 mL lavender top EDTA tubes. Place pre-printed "**PLA**" Cryovial Labels on the (12) 2.0 mL cryovial with purple-caps and (1) 2.0 mL cryovial with blue-cap (if necessary, for residual). Place pre-printed "**BC**" Cryovial Tube Labels on the (4) 2.0 mL cryovials with gray-caps. The (8) 0.75 mL cryovials with purple-caps will not be labeled and are pre-etched with the specimen type.

2. Using a blood collection set and a holder, collect blood into the **EDTA (Lavender-Top) Blood Collection Tubes (10 mL)** using your organization's recommended procedure for standard venipuncture technique.
    a. **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 the last collection tube.
        d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

3. 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.

4. 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.

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

6. Preferably within 30 minutes of blood collection, centrifuge balanced tubes for 10 minutes at 2000 x g room temperature. **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.)**

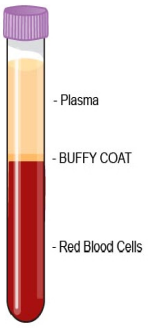
7. While centrifuging, remember to record all times, temperatures and spin rates

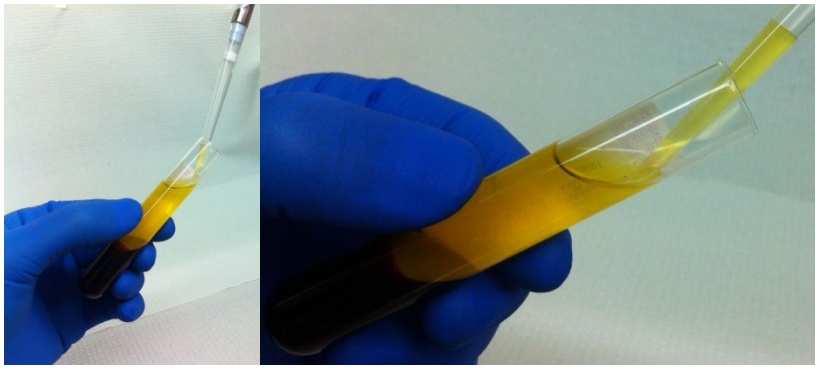
on the Biological Sample and Shipment Notification Form.

8. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.

9. Record time aliquoted on the Biological Sample Shipment and Notification Form.

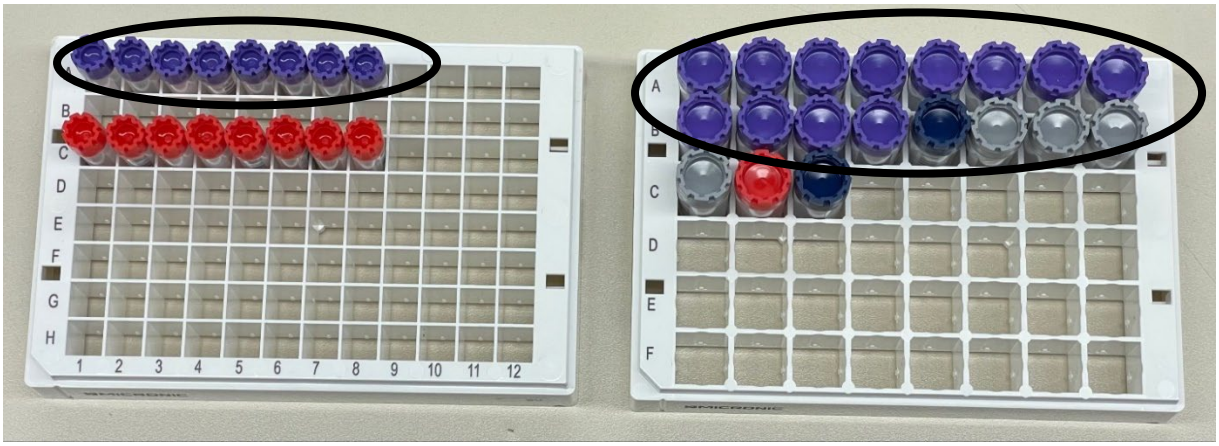
10. Remove the plasma, being careful not to agitate the packed red blood cells at the bottom of the tube. Tilt the tube and place a disposable pipette tip along the lower side of the wall without touching the pellet (buffy coat) so that plasma is not contaminated (see below). Transfer plasma from all four EDTA tubes into the 50 mL conical tube and gently invert 3 times. Aliquot 0.25 mL per 0.75 mL cryovial first, and aliquot the remaining plasma in 1.5 mL quantities per 2.0 mL cryovial (total vials = 8 with 0.25 mL each, 12 with 1.5 mL, and 1 residual with <1.5 mL per participant). Each EDTA tube should yield, on average, 5 mL of plasma. Be sure to only place **plasma** in cryovials with purple-caps and labeled with “PLA” labels**.** Take caution not to disturb the red blood cells at the bottom of the tube. If there is extra plasma left, use 1 blue-capped cryovial with plasma label for another <1.5 mL aliquot of plasma and label as appropriate. **If a residual aliquot (<1.5 mL) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**





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

Purple-Top Plasma Aliquots (up to 21 possible, including the residual)

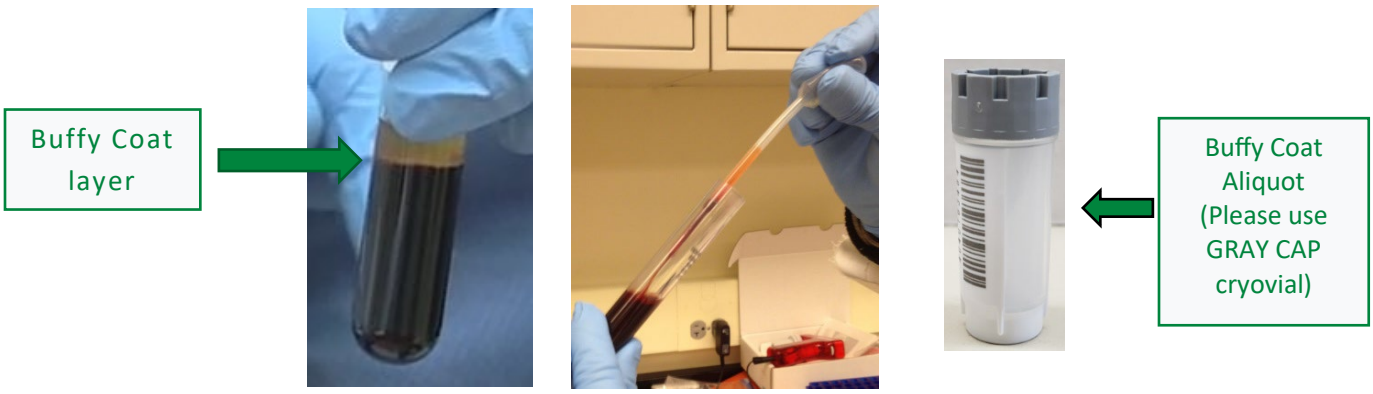


Close up view of Purple-Top 0.75 mL Micronic™ cryovial

Close up view of Purple-Top 2.0 mL Micronic™ cryovial

11. Place the (8) 0.75 mL labeled cryovials in the 96 cell cryobox and up to (13) 2.0 mL labeled cryovials in the 48 cell cryobox and place on pelleted dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on pelleted dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample Shipment and Notification Form.

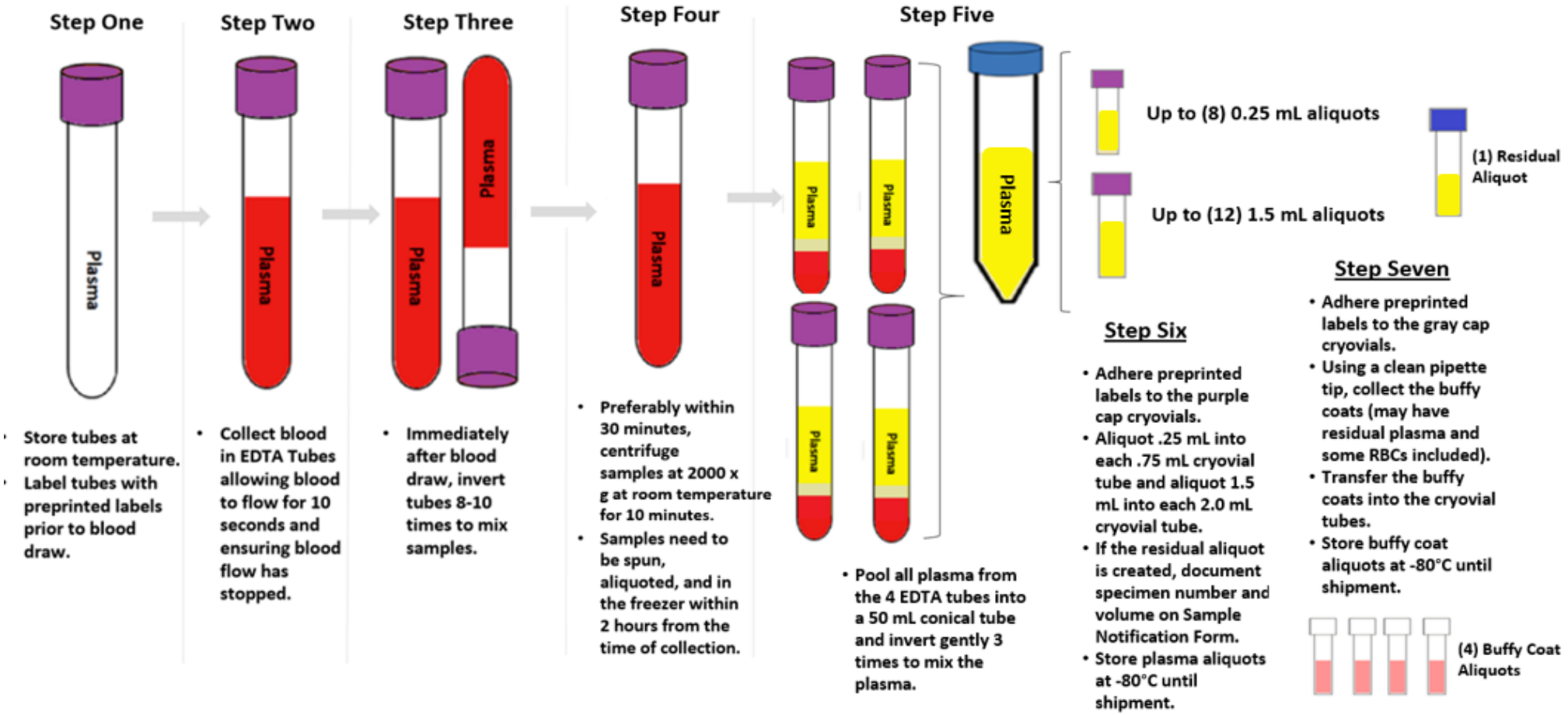
12. After plasma has been removed from the EDTA (Lavender-Top) Blood Collection Tubes (10 mL), aliquot the buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) from one EDTA tube into a labeled, gray-capped cryovial using a micropipette. The buffy coat aliquot is expected to have a reddish color from the RBCs. Aliquot each buffy coat into a separate cryovial. Repeat this step for each EDTA tube, placing these buffy coats into each subsequent gray-capped cryovial with “BUFFY COAT” label.



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

14. Place the (4) 2.0 mL labeled buffy coat cryovials in the 48 cell cryobox and place on pelleted dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on pelleted dry ice

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



Important Note: Ensure all tubes are not expired prior to collection and processing of samples.

6.4 2.5 mL PAXgene™ Tube for RNA x 2

Whole Blood Collection for Isolation of RNA: 2.5 mL PAXgene™ RNA Tube

Important Note: Ensure all tubes are not expired prior to collection and processing of samples.

1. **CRITICAL STEP: Store PAXgene™ RNA Tubes at room temperature (64^{0}F - 77^{0}F or 18°C to 25°C) before use.**

2. Place completed Site and CADASIL ID Label and "**WBLD RNA – PAX10**" Collection Tube Label on the PAXgene™ tubes prior to blood draw; no processing is required for this tube**. Both tubes are to be shipped to NCRAD frozen, without processing at the collection site.**

3. Using a blood collection set and a holder, collect blood into the **PAXgene™ RNA Tube** using your organization's recommended procedure for standard venipuncture technique.
    a. **The following techniques shall be used to prevent possible backflow:**
        i. Place participant's arm in a downward position.
        ii. Hold tube in a vertical position, below the participant's arm during blood collection.
        iii. Release tourniquet as soon as blood starts to flow into last collection tube.
        iv. 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 the tube before removing the tube from the holder.** The PAXgene™ RNA Tube with its vacuum is designed to draw 2.5 mL of blood into the tube.

5. **Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene™ RNA Tube 8 – 10 times**.

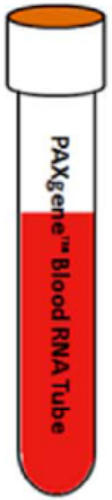
6. Place the PAXgene™ RNA tube upright in a **WIRE** rack and transfer the PAXgene™ RNA tube to a **-80°C freezer.** Keep the **PAXgene™ RNA Tube in -80°C freezer** for storage until you ship on pelleted dry ice to NCRAD. Complete remainder of the Biological Sample and Shipment Notification Form (Appendix B).

RNA Preparation (2.5ml PAXgene™ Tube) X 2

Step One

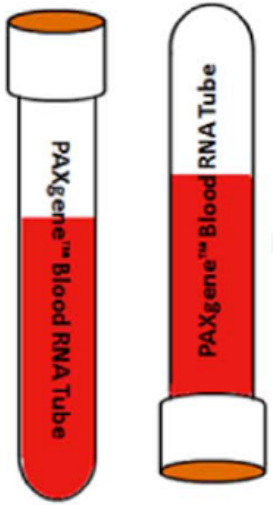
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



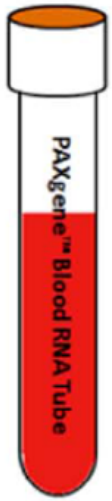
- Collect blood in PAXgene™ tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four



- Store tubes at -80°C in a wire rack until shipment.

Important Note: Ensure all tubes are not expired prior to collection and processing of samples.

6.5 SST (Tiger-Top) Blood Collection Tubes (8.5 mL) for Serum x 1

Whole Blood Collection for Isolation of Serum: SST (Tiger-Top) Blood Collection Tube (8.5 mL) (for processing of serum aliquots)

> **Important Note:** Ensure all tubes are not expired prior to collection and processing of samples.

1. Place completed Site and CADASIL ID Label and **"WBLD SST8.5"** Collection Tube Label (with "COLLECT" under the study name) on the SST (Tiger-Top) Blood Collection Tube (8.5 mL). Place pre-printed "**SER**" Cryovial Tube Labels on the (1) 2.0 mL cryovials with red-caps and (1) 2.0 mL cryovial with blue-cap (if necessary, for residual). The (8) 0.75 mL cryovials with red-caps will not be labeled and are pre-etched with the specimen type.

2. Using a blood collection set and a holder, collect blood into **SST (Tiger-Top) Blood Collection Tube (8.5 mL)** using your organization's recommended procedure for standard venipuncture technique
    a. **The following techniques shall be used to prevent possible backflow:**
        i. Place participant's arm in a downward position.
        ii. Hold tube in a vertical position, below the participant's arm during blood collection.
        iii. Release tourniquet as soon as blood starts to flow into last collection tube.
        iv. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.

3. 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 8.5 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.

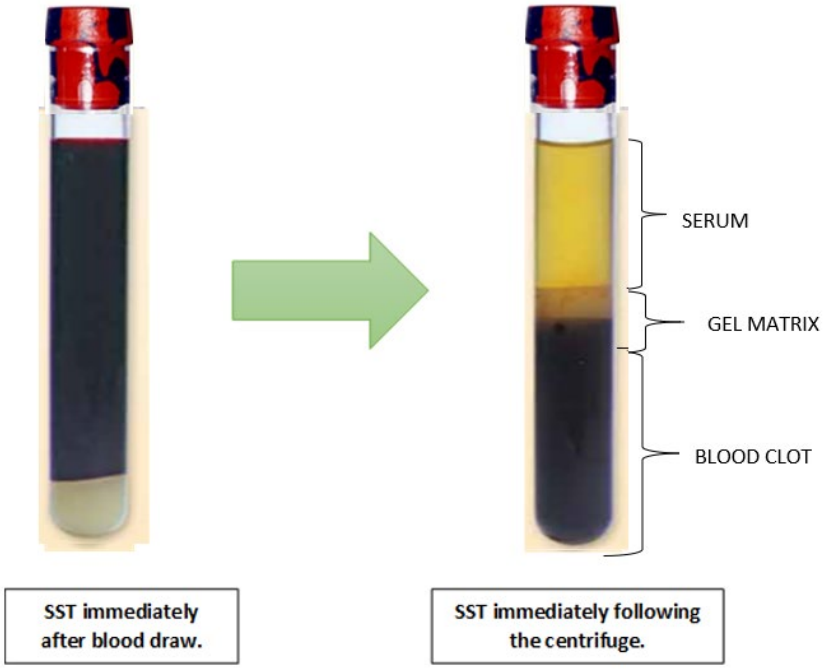
4. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) each tube 5 times.**

5. **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 sit 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.**

6. After 30-60 minutes of clotting, centrifuge the collection tube for 10 minutes at 2000 x g at room temperature. **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).**

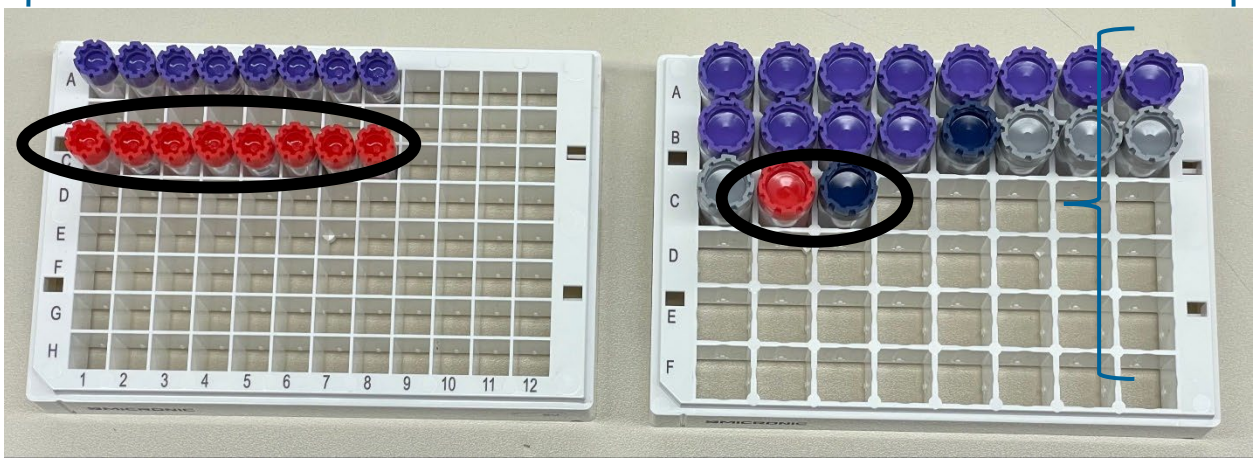
7. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form Appendix B.
    a. Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.

8. Record time aliquoted on the Biological Sample Shipment and Notification Form.



9. 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 0.25 mL per 0.75 mL cryovial first, and aliquot the remaining serum in 1.5 mL quantities per 2.0 mL cryovial (total vials = 8 with 0.25 mL each, 1 with 1.5 mL, and 1 residual with <1.5 mL per participant). The Serum tube should yield, on average, 4-5 mL of serum. Be sure to only place **serum** in cryovials labeled with the “SER” label and red caps. If there is extra serum left, use 1 blue-capped cryovial with serum label for another <1.5 mL aliquot of serum and label as appropriate. **If a residual aliquot (<1.5 mL) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**

Red-Top Serum Aliquots (up to 10 possible, including the residual)

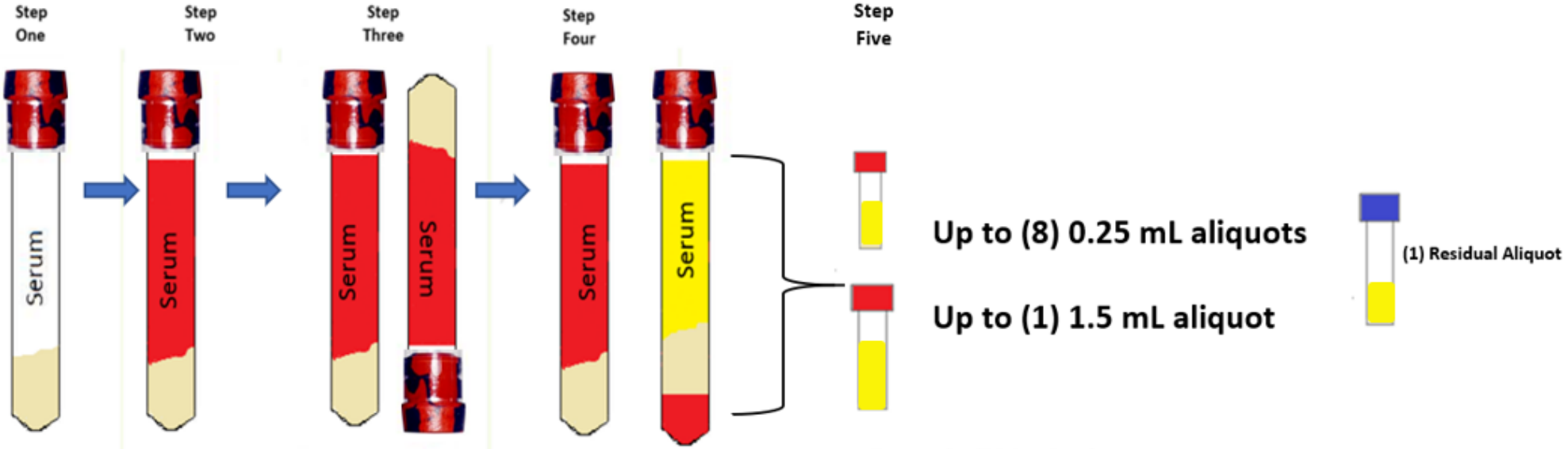


Close up view of Red-Top 0.75 mL Micronic™ cryovial

Close up view of Red-Top 2.0 mL Micronic™ cryovial

10. Place the (8) 0.75 mL labeled serum cryovials in the 96 cell cryobox and up to (2) 2.0 mL labeled cryovials in the 48 cell cryobox and place on pelleted dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on pelleted dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

SST (Tiger-Top) Blood Collection Tubes (8.5 mL) for Serum x 1



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

- **Collect blood in (1) 8.5 Tiger-Top tube allowing blood to flow for 10 seconds and ensure blood flow has stopped.**

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

- Allow blood to clot for 30 minutes.
- Within 2 hours of blood draw, centrifuge samples at 2000 x g at **room temperature for 10 minutes.**

- Adhere preprinted labels to the red-cap cryovials.
- Aliquot 0.25 mL into each 0.75 mL cryovial tube and aliquot 1.5 mL into the 2.0 mL cryovial tube.
- If a residual aliquot is created, document specimen number on Sample Notification Form.
- Store serum aliquots at -80°C until shipment.

Important Note: Ensure all tubes are not expired prior to collection and processing of samples.

7.0 Incomplete or Difficult Blood Draws and 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.

Situations may arise that prevent study coordinators from obtaining the total amount scheduled for biofluids. In these situations, please follow the below steps:

1. If the biofluids 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 biofluids at a scheduled visit **are not** collected:
    a. Contact the CADASIL Global Coordinator and a NCRAD coordinator to alert them of the challenging blood draw or circumstances as to why biofluids were not collected.
    b. Schedule participant for a re-draw visit as quickly as possible.

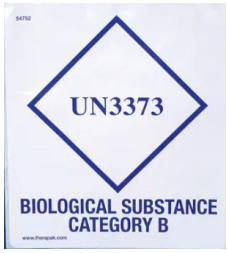
8.0 Packaging and Shipping Instructions

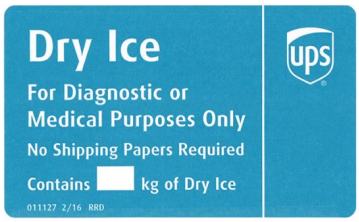
Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/Aliquoting	Tubes to NCRAD	Ship
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	**EDTA (Lavender-Top) Blood Collection Tube (10 mL)**	**4**	**N/A**	**N/A**	**N/A**
	PLASMA: 0.75 mL cryovials	8	0.25 mL plasma aliquot per 0.75 mL cryovial (Micronic™ purple cap)	8	**Frozen**
	PLASMA: 2.0 mL cryovials	12	1.5 mL plasma aliquot per 2.0 mL cryovial (Micronic™ purple cap)	12	
	PLASMA RESIDUAL: 2.0 mL cryovials	1	1.5 mL plasma aliquot per 2.0 mL cryovial (Micronic™ blue cap)	1	
	BUFFY COAT: 2.0 mL cryovials	4	1 mL buffy coat aliquot per 2.0 mL cryovial (Micronic™ gray cap)	4	
Whole blood for RNA extraction	**PAXgene™ Blood Collection Tube (2.5 mL)**	**2**	**N/A**	**2**	**Frozen**
Whole blood for isolation of serum	**SST (Tiger-Top) Blood Collection Tubes (8.5 mL)**	**1**	**N/A**	**N/A**	**N/A**
	SERUM: 0.75 mL cryovials	8	0.25 mL serum aliquot per 0.75 mL cryovial (Micronic™ red cap)	8	**Frozen**
	SERUM: 2.0 mL cryovials	1	1.5 mL serum aliquot per 2.0 mL cryovial (Micronic™ red cap)	1	
	SERUM RESIDUAL: 2.0 mL cryovials	1	1.5 mL serum aliquot per 2.0 mL cryovial (Micronic™ blue cap)	1	

ALL study personnel responsible for shipping should be certified in biofluid shipping (i.e., IATA certification). If not available at your organization, please contact NCRAD with questions and information regarding resources.

Packing and Labeling Guidelines:

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





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

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.

Large Frozen Shipper:

** 45 lbs of dry ice pellets

AND

- Fits up to 8 x 48-slot cryoboxes

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

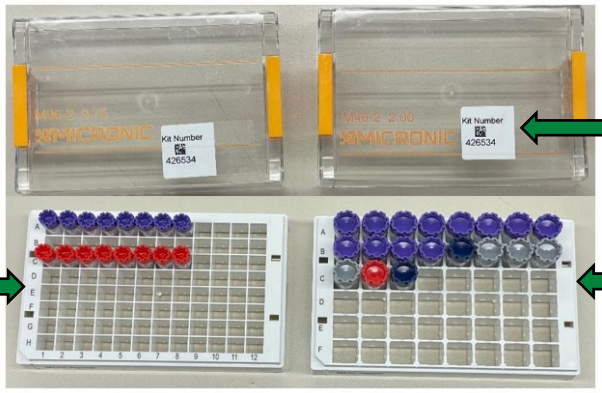
Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary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

8.1 Frozen Packaging Instructions

1. On the day of scheduled pick-up, begin packaging specimens on pelleted dry ice **~1 hour before UPS arrives**. Hold samples in -80°C freezer until it is time to package the specimens on pelleted dry ice for shipment. If storage in a -80°C freezer until UPS pick-up is not possible, package shipments no more than 4 hours before the expected pick-up time.
    1. **Important Note:** If shipping samples same day of collection, place samples upright on pelleted dry ice for 2 hours before shipment to ensure samples are completely frozen.

2. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu. Attach the following to the email:
    a. Completed Biological Sample and Shipment Notification Form to the email notification.
    (See Appendix B for an example of the NCRAD sample form)
    b. If email is unavailable, please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.

3. Place all frozen labeled aliquots of plasma, buffy coat, serum, and residual aliquots from the same participant in the cryovial cryobox.
    i. Each 96 cell cryobox will hold approximately 24 cryovial samples.
        i. Place plasma and serum within one cryobox (8 plasma and 8 serum per participant blood draw).
    ii. Each 48 cell cryobox will hold approximately 26 cryovial samples.
        i. Place plasma, buffy coat, serum, and residuals within one cryobox (13 plasma, 4 buffy coat, and 2 serum per participant blood draw).
    iii. Each pair of cryoboxes should contain the specimens from the same patient, per time point.
    d. Batch shipping should be performed every (3) three months or when specimens from 4 participants accumulate, whichever is sooner.



4. Label the outside of the cryoboxes with the appropriate kit number label. Place the cryoboxes with samples from the same participant into one clear biohazard bag (do NOT remove the absorbent material found in the bag).
5. Place frozen (2) PAXgene™ tubes in provided bubble wrap tube sleeves, seal, and place in biohazard bag with cryoboxes. Seal biohazard bag according to the instructions on the bag.

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

7. Place the biohazard bag into the provided Styrofoam-lined shipping container on top of the pelleted dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container (as pictured below).

8. Fully cover the cryoboxes and tubes with approximately 2 inches of pelleted dry ice.

9. The inner Styrofoam shipping container must contain approximately 30-45 lbs. (or 21kg) of pelleted dry ice. The pelleted dry ice should entirely fill the inner box to ensure the frozen state of the specimens.

Full Shipping Container with Batched Samples and Pelleted Dry Ice

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

11. Complete the UPS Dry Ice Label
    1. Net weight of pelleted dry ice in kg (must match amount on the airbill)
    2. 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 label to the outside of package, taking care not to overlap labels.

IMPORTANT!
Complete the UPS Dry Ice label or UPS may reject or return your package.

13. Specimens should be sent to the below address via **UPS Next Day Air**. Frozen shipments should be sent **Monday through Wednesday** to avoid shipping delays on Thursday or Friday. UPS does not replenish dry ice if shipments are delayed or held over during the weekend.

NCRAD
351 West 10th Street
TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

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.

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

For frozen shipments, include no more than eight cryovial boxes per shipping container in order to have room for a sufficient amount of pelleted dry ice to keep samples frozen up to 24 hours. Each biohazard bag should contain 2 cryovial boxes and 2 PAXgene™ tubes from the same participant.

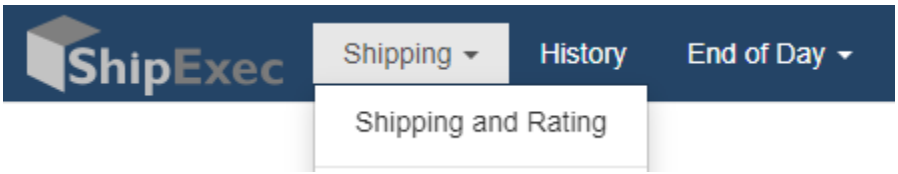
SHIP ALL FROZEN SAMPLES MONDAY - WEDNESDAY ONLY!
BE AWARE OF HOLIDAYS!!
BE AWARE OF INCLEMENT WEATHER THAT MAY DELAY SHIPMENT/DELIVERY OF SAMPLES

Remember to complete the Biological Sample and Shipment Notification Form (Appendix B and 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.

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

8.2 Frozen Shipping Instructions

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

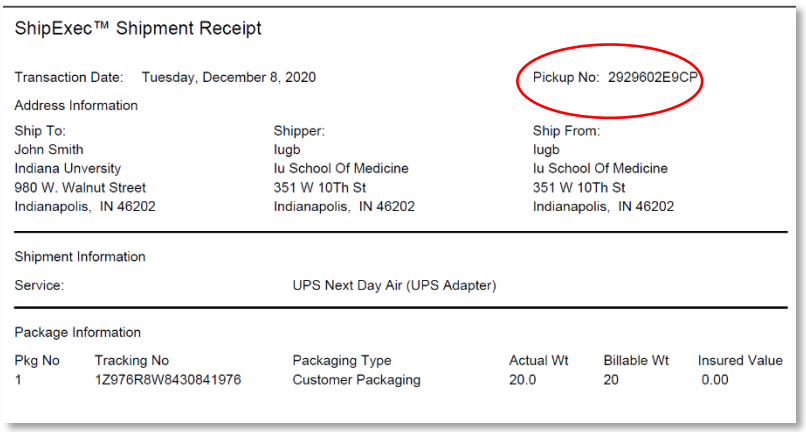


2. Click “Shipping” at the top of the page and select “Shipping and Rating”
3. Select your study from the “Study Group” drop down on the right side of the main screen. Choosing your study will automatically filter the address book to only addresses within your study.
4. Click on the magnifying glass icon in the “Ship From” section to search for your shipping address.

    i. Search by Company (site), Contact (name), or Address 1 (first line of your site's street address). Click Search.
    ii. Click Select to the left of the correct contact information.
5. Verify that both the shipping information AND study reference are correct for this shipment.
    a. If wrong study contact or study reference, click Reset in the bottom right of the screen to research for the correct information.
6. Enter Package Information
    a. Frozen shipments
        i. Enter the total weight of your package in the "Weight" field.
        ii. Enter the dry ice weight in the "Dry Ice Weight" field.
        iii. If the "Dry Ice Weight" field is higher than the "Weight" field, you will receive an error message and need to re-enter these values.
    b. Click "Ship" in the bottom right of the page when complete.
7. If your site does not already have a daily UPS pickup, you will need to schedule one.
    a. Click the blue "Pickup Request" button. Enter the earliest pickup time and latest pickup time in 24-hr format.
        i. Users must schedule pickup **minimum** 1 hour before "Earliest Time Ready"
    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.
        i. Room number field is free text, Floor field is numerical only.
    d. Click Save.
    e. Click on "Ship" button in the bottom right corner when ready to ship the package.
8. Print the airbill that is automatically downloaded.
    a. To reprint airbill, click History at the top left of the page.
    b. Click "Detailed Report" from the dropdown menu on the right side of the page.
    c. Enter tracking number if known. Otherwise, search by ship date. Click Search.
    d. 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. A UPS Pickup is automatically scheduled at the address you are shipping from, and the pickup is charged to NCRAD.
12. If shipment occurs too late in the day for an automatic UPS pickup, you will receive an email stating that the pickup could not be scheduled, and you will need to make other arrangements.

Note:

- The “Pickup No:” is the reference number to your specific pickup request in case there are any issues with your package being picked up by UPS.
- Check Pickup details by going to UPS.com, click on the Shipping, select Schedule a Pickup, and look on the right side of screen to click on “Pickup Status”. Enter in the Pickup No. listed on receipt into PRN field and submit



ShipExec™ Shipment Receipt

Transaction Date: Tuesday, December 8, 2020

Pickup No: 2929602E9CP

Address Information

Ship To:	Shipper:	Ship From:
John Smith	Iugb	Iugb
Indiana Unversity	Iu School Of Medicine	Iu School Of Medicine
980 W. Walnut Street	351 W 10Th St	351 W 10Th St
Indianapolis, IN 46202	Indianapolis, IN 46202	Indianapolis, IN 46202

Shipment Information

Service: UPS Next Day Air (UPS Adapter)

Package Information

Pkg No	Tracking No	Packaging Type	Actual Wt	Billable Wt	Insured Value
1	1Z976R8W8430841976	Customer Packaging	20.0	20	0.00

9.0 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.

The COINS (Collaborative Informatics and Neuroimaging Suite) Data Collection team at TReNDS will be collaborating with NCRAD to reconcile information captured in their database compared to samples received and logged at NCRAD. Information that appears incorrect in the COINS Data Portal 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 monthly.
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 ATRI 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 List

Appendix A: Rate of Centrifugation Worksheet

Please complete and return this form by email to the NCRAD Coordinator 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.

Appendix B: Biological Sample and Shipment Notification Form

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

UPS tracking #: 1Z976R8W Date: _____

From: _____ Phone: _____ Email: _____

Study: CADASIL Site ID: _____ CADASIL IND #: CC

Sex: M F Year of Birth: _____

Visit: Baseline 18 Month 36 Month

KIT LABEL/BARCODE

Blood Collection:

Date of Draw:	[MMDDYY]	Time of Draw:	[HHMM]
Date subject last ate:	[MMDDYY]	Time subject last ate:	[HHMM]

RNA (PAXgene™ Tubes)						
#1	Specimen Number (Last four digits):	_____	Original volume drawn:	_____ml	Time PAXgene™ tubes placed in freezer:	_____[HHMM]
#2	Specimen Number (Last four digits):	_____	Original volume drawn:	_____ml		

Blood Processing:

Serum (Red-top) Tube (8.5 mL)			
Time spin started:	_____[HHMM]	Number of 0.25 mL serum aliquots created (red cap):	_____
Duration of centrifuge:	_____Mins	Number of 1.5 mL serum aliquots created (red cap):	_____
Temp of Centrifuge:	_____°C	Volume of residual serum aliquot (less than 1.5 mL in blue cap):	_____mL ☐ N/A
Rate of centrifuge:	_____x g	Specimen number of residual serum aliquot (last four digits):	_____ ☐ N/A
Original volume drawn:	_____mL	Time aliquots placed in freezer:	_____[HHMM]
Time aliquoted:	_____[HHMM]	Storage temperature in freezer:	_____°C

Plasma & Buffy Coat (Lavender-top) Tubes (10 mL)			
Time spin started:	_____[HHMM]	Time aliquoted:	_____[HHMM]
Duration of centrifuge:	_____Mins	Number of 0.25 mL plasma aliquots created (purple cap):	_____
Temp of Centrifuge:	_____°C	Number of 1.5 mL plasma aliquots created (purple cap):	_____
Rate of centrifuge:	_____x g	Volume of residual plasma aliquot (less than 1.5 mL in blue cap):	_____mL ☐ N/A
Original volume drawn - EDTA #1	_____mL	Specimen number of residual plasma aliquot (last four digits):	_____ ☐ N/A
Original volume drawn - EDTA #2	_____mL	Time aliquots placed in freezer:	_____[HHMM]
Original volume drawn - EDTA #3	_____mL	Storage temperature in freezer:	_____°C
Original volume drawn - EDTA #4	_____mL		
Aliquot volume – Buffy coat #1	_____mL	Buffy coat aliquot #1 (last four digits):	_____
Aliquot volume – Buffy coat #2	_____mL	Buffy coat aliquot #2 (last four digits):	_____
Aliquot volume – Buffy coat #3	_____mL	Buffy coat aliquot #3 (last four digits):	_____
Aliquot volume – Buffy coat #4	_____mL	Buffy coat aliquot #4 (last four digits):	_____

NOTES:

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