V05.2025

Section	Change
Document Footer	The version date was updated for this amendment.
Throughout Document	Minor changes made to phrasing and wording. No alterations to procedures were made.
Throughout Document	Updated logo and format
3.1	Updated contact information

Manual of Procedures

National Cell Repository for Alzheimer's Disease (NCRAD):

A Phase 1b, 12-Month, Randomized, Double-Blind, Placebo-Controlled Study of the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Preliminary Efficacy of Salsalate in Patients with Mild to Moderate Alzheimer's Disease.

SAL-AD

Biospecimen Collection, Processing, and Shipment Manual

University of California
San Francisco

TABLE OF CONTENTS

1.0 ABBREVIATIONS

AC-HIS	Acetylated Histone
AD	Alzheimer's Disease
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
LP	Lumbar Puncture
NCRAD	National Cell Repository for Alzheimer's Disease
PK	Pharmacokinetics
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
SAL	Salsalate
WBC	White Blood Cells

2.0 PURPOSE

The purpose of this manual is to provide SAL-AD 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 the SAL-AD study visits. It includes instructions for biospecimen submission to the National Cell Repository for Alzheimer's Disease (NCRAD) located at Indiana University.

The following samples may be collected at study visits:

- Plasma (ac-his, PK, banking)
- Buffy Coat (DNA extraction)
- Serum (banking)
- CSF (banking)

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 specimens to be submitted to NCRAD for the SAL-AD 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

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

3.2 Hours of Operation

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

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

For packing and shipment details of both ambient and 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 Holiday Schedules

Please note: 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 Holiday Observations

Date	Holiday
January 1	New Year's Day
3rd Monday in January	Martin Luther King, Jr Day
4th Monday in May	Memorial Day
July 4	Independence Day (observed)
1st Monday in September	Labor Day
4th Thursday in November	Thanksgiving
4th Friday in November	Friday after Thanksgiving
December 25	Christmas Day

Please note that between December 20th 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 between December 20th and January 2nd. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 20th by e-mailing alzstudy@iu.edu, so that they can arrange to have staff available to process incoming samples.

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

4.0 SAL-AD 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 (for CSF only)
- Wet ice (for CSF only)

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 ≥ 2,000 rcf (2,000 x g) at room temperature and ≥1,500 rcf (1,500 x g) with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, site must provide:

- Dry ice (approximately 30-45 lbs per shipment)
- Scale to weigh shipment boxes

4.2 Biospecimens Sent to NCRAD

Biospecimens collected include whole blood and CSF. Please refer to the below table for the biospecimen schedule.

	Screen	Baseline	6 month	12 month
DNA (Buffy Coat)		x		
Plasma (ac-his)	x		x	x
Plasma (PK)			x	x
Plasma (banking)		x	x	x
Serum (banking)		x	x	x
CSF	x		x	x

Whole blood will be collected in up to three different collection tubes (6 ml Purple-top EDTA tube, 10 ml Purple-top EDTA tube, and 10 ml red-top serum determination tube). The Purple -top EDTA tubes are processed locally into fractions, aliquoted, frozen at the study site, and then shipped to NCRAD. The red-top serum determination tube is processed locally into serum fractions, aliquoted, frozen at the study site, and then shipped to NCRAD.

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

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

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

4.3 Biospecimen Collection Charts

4.3.1 Biospecimen Collection: Screening

Sample Type	Container Type	Number Supplied in Kit	Aliquot Volume	Number sent to NCRAD	Ship
Whole blood for isolation of plasma	**6 ml EDTA (Purple-Top) Blood Collection Tube for ac-his**	**1**	**N/A**	**N/A**	**N/A**
	PLASMA: 0.5 ml Siliconized Cryovials with a clear cap and purple sticker (residual volume placed in 0.5 ml clear cap cryovial with blue sticker)	13 cryovials: 12 with PURPLE sticker Caps, 1 with BLUE sticker Cap	0.25 ml plasma aliquots per 0.5 ml Siliconized Cryovial	8-12	Frozen
CSF	**50 ml screw top centrifuge tubes with blue caps**	**2**	**N/A**	**N/A**	**N/A**
	2 ml Siliconized Cryovials with a clear cap and white sticker (residual volume placed in 2 ml clear cap cryovial with blue sticker)	41 cryovials: 40 clear cap with WHITE stickers, 1 clear cap with BLUE sticker	0.5 ml CSF aliquots per 2 ml Siliconized Cryovials	40	Frozen

* Please refer to the table in Section 4.2 for another view of the specimen collection schedule.

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

4.3.2 Biospecimen Collection: Baseline

Sample Type	Container Type	Number Supplied in Kit	Aliquot Volume	Number sent to NCRAD	Ship
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	**10 ml EDTA (Purple-Top) Blood Collection Tube**	**1**	**N/A**	**N/A**	**N/A**
	PLASMA: 0.5 ml Siliconized Cryovials with a clear cap and Purple sticker (residual volume placed in 0.5 ml clear cap cryovial with blue sticker)	21 cryovials: 20 clear cap with PURPLE sticker, 1 clear cap with BLUE sticker	0.25 ml plasma aliquot per 0.5 ml siliconized cryovial	16-20	Frozen
	BUFFY COAT: 2 ml cryovial with a clear cap	1	1 ml buffy coat aliquot per 2 ml cryovial	1	Frozen
Whole blood for isolation of serum	**10 ml Serum Determination (Red-Top) Blood Collection Tube**	**1**	**N/A**	**N/A**	**N/A**
	SERUM: 0.5 ml Siliconized Cyrovials with a clear cap and red sticker (residual volume placed in 0.5 ml clear cap cryovial with blue sticker)	21 cryovials: 20 clear cap with RED sticker, 1 clear cap with BLUE sticker	0.25 ml serum aliquot per 0.5 ml siliconized cryovial	16-20	Frozen

4.3.3 Biospecimen Collection: 6 month and 12 month

Sample Type	Container Type	Number Supplied in Kit	Aliquot Volume	Number sent to NCRAD	Ship
Whole blood for isolation of plasma for ac-his, PK, and plasma banking	**6 ml EDTA (Purple-Top) Blood Collection Tube for ac-his and PK**	**2**	**N/A**	**N/A**	**N/A**
	10 ml EDTA (Purple-Top) Blood Collection Tube for plasma banking	**1**	**N/A**	**N/A**	**N/A**
	PLASMA: 0.5 ml Siliconized Cryovials with a clear cap and Purple sticker (residual volume placed in 0.5 ml clear cap cryovial with blue sticker)	47 cryovials: 44 clear cap with PURPLE sticker, 3 clear cap with BLUE sticker	0.25 ml plasma aliquots per 0.5 ml siliconized cryovial	16-20 plasma; 8-12 plasma PK; 8-12 plasma ac-his	Frozen
Whole blood for isolation of serum	**10 ml Serum Determination (Red-Top) Blood Collection Tube**	**1**	**N/A**	**N/A**	**N/A**
	SERUM: 0.5 ml Siliconized Cyrovials with a clear cap and red sticker (residual volume placed in 0.5 ml clear cap cryovial with blue sticker)	21 cryovials: 20 clear cap with RED sticker, 1 clear cap with BLUE sticker	0.25 ml serum aliquot per 0.5 ml siliconized cryovial	16-20	Frozen
CSF	**50 ml screw top centrifuge tubes with blue caps**	**2**	**N/A**	**N/A**	**N/A**
	2 ml Siliconized Cryovials with a clear cap and white sticker (residual volume place in 2 mL clear cap cryovial with blue sticker)	41 cryovials: 40 clear cap with WHITE Stickers, 1 clear cap with BLUE stickers	0.5 ml CSF aliquots per 2 ml siliconized cryovials	40	frozen

5.0 Specimen Collection Kits, Shipping Kits, and Supplies

Research specimen collection kits as well as clinical lab supplies (except aforementioned equipment supplies) will be provided by NCRAD. These materials include blood tubes, boxes for plasma/buffy coat/CSF aliquots, and partially completed shipping labels to send biological materials to NCRAD. Barcoded kit labels, site and subject ID labels, collection tube labels, and aliquot tube labels will also be provided by NCRAD. Collection tube labels 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 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.*

SAL-AD Screening Visit Blood Kit

Quantity	Screening Blood Kit Components
1	EDTA (Purple-Top) Blood Collection Tube (6 ml)
12	Siliconized Cryovial (0.5 ml): Clear Cap with Purple Sticker
1	Siliconized Cryovial (0.5 ml): Clear Cap with Blue Sticker
13	Labels for blood collection tubes and aliquots
2	Labels with kit number
2	Labels for handwritten Site and subject ID
1	95 kPa Biohazard bag (large)
1	100 ml Absorbent Sheet
1	Resealable bag
1	81 Cell Cryovial Box

Screening CSF Kit

Quantity	NCRAD CSF Kit Components
40	Siliconized Cryovial (2 ml): Clear Cap with White Sticker
1	Siliconized Cryovial (2 ml): Clear Cap with Blue Sticker
2	Individually Packaged Sterile 50 ml Conical Tube
40	Labels for CSF collection tubes and aliquots
3	Labels with kit number

SAL-AD Baseline Blood Kit

Quantity	Baseline Blood Kit Components
1	EDTA (Purple-Top) Blood Collection Tube (10 ml)
1	Serum (Red-Top) Blood Collection Tube (10 ml)
20	Siliconized Cryovial (0.5 ml): Clear Cap with Purple Sticker
20	Siliconized Cryovial (0.5 ml): Clear Cap with Red Sticker
2	Siliconized Cryovial (0.5 ml): Clear Cap with Blue Sticker
1	Cryovial (2 ml): Clear Cap with No Sticker
1	95 kPa Large Biohazard Bag
1	Resealable bag
1	100 ml Absorbent Sheet
43	Labels for blood collection tubes and aliquots
2	Labels with kit number
3	Labels for handwritten Site and subject ID
1	81 Cell Cryovial Box

SAL-AD 6 & 12 Month Visit Blood Kit

Quantity	6 & 12 Month Visit Blood Kit Components
2	EDTA (Purple-Top) Blood Collection Tube (6 ml)
1	EDTA (Purple-Top) Blood Collection Tube (10 ml)
1	Serum (Red-Top) Blood Collection Tube (10 ml)
44	Siliconized Cryovial (0.5 ml): Clear Cap with Purple Sticker
20	Siliconized Cryovial (0.5 ml): Clear Cap with Red Sticker
4	Siliconized Cryovial (0.5 ml): Clear Cap with Blue Sticker
68	Labels for blood collection tubes and aliquots
2	Labels with kit number
5	Labels for handwritten Site and subject ID
1	95 kPa Large Biohazard Bag
1	Resealable bag
1	100 ml Absorbent Sheet
1	81 Cell Cryovial Box

CSF Kit (CSF at 6 & 12 Month Visits)

Quantity	NCRAD CSF Kit Components
40	Siliconized Cryovial (2 ml): Clear Cap with White Sticker
1	Siliconized Cryovial (2 ml): Clear Cap with Blue Sticker
2	Individually Packaged Sterile 50 ml Conical Tube

40	Labels for blood collection tubes and aliquots
4	Labels with kit number
1	95 kPa Large Biohazard Bag
1	Resealable bag
1	100 ml Absorbent Sheet
1	81 Cell Cryovial Box

Frozen Shipping Supply Kit

Quantity	Frozen Shipping Kit Components
1	FedEx return airbill and pouch
1	Shipping box/Styrofoam container
1	Warning label packet with dry ice sticker

SAL-AD Supplemental Supply Kit

One SAL-AD Supplemental Supply Kit will be sent to all sites with the initial shipment of kit materials unless otherwise specified.

Quantity	SAL-AD Supplemental Kit Components
5	81 Cell Cryovial Box
5	95 kPa Large Biohazard Bag
5	100 ml Absorbent Sheet
25	Siliconized Cryovial (0.5 ml): Clear Cap with Purple Sticker
25	Siliconized Cryovial (0.5 ml): Clear Cap with Red Sticker
25	Siliconized Cryovial (0.5 ml): Clear Cap with Blue Sticker
25	Siliconized Cryovial (2 ml): Clear Cap with White Sticker
25	Siliconized Cryovial (2 ml): Clear Cap with Blue Sticker
25	Cryovial (2 ml): Clear Cap with No Sticker
5	FedEx return airbill
15	Individually Packaged Sterile 50 ml Conical Tube
5	EDTA (Purple-Top) Blood Collection Tube (10 ml)
10	EDTA (Purple-Top) Blood Collection Tube (6 ml)
5	Serum (Red-Top) Blood Collection Tube (10ml)
5	Warning Label Packet

Individual Supplies

Available upon request within the kit request module.

Quantities	Item
5, 10	Cryovial box (holds up to 81 cryovials)
10, 25	Siliconized Cryovial (0.5 ml): Clear Cap with Purple Sticker
10, 25	Siliconized Cryovial (0.5 ml): Clear Cap with Red Sticker
25, 50	Siliconized Cryovial (2 ml): Clear Cap with White Sticker
25, 50	Siliconized Cryovial (0.5 ml): Clear Cap with Blue Sticker
25, 50	Cryovial tube (2 ml): Clear Cap with No Sticker
5, 10	FedEx return airbill
By Request	Shipping container for frozen shipment (shipping and Styrofoam box)
5, 10	Individually Packaged Sterile 50ml Conical Tube
15, 30	Sterile screw-top conical tube with blue cap (50 ml)
5, 10	Screw-top conical tube with blue cap (15 ml)
5, 10	95 kPa Large Biohazard Bag
5, 10	100 ml Absorbent Sheet
5, 10, 15	EDTA (Purple-Top) Blood Collection Tube (10 ml)
5, 10, 15	EDTA (Purple-Top) Blood Collection Tube (6 ml)
5, 10, 15	Serum (Red-Top) Blood Collection Tube (10ml)
5, 10	Warning label packet
By Request	Subject ID label

5.2 Kit Supply to Study Sites

Each individual site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits.

Visit kits.iu.edu/salad to request additional kits and follow the prompts to request the desired supplies. Options include ordering specific number of kits or individual supplies.

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

6.0 BLOOD COLLECTION AND PROCESSING PROCEDURES

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

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

At the screening visit, ONLY draw the following:

1. **6 ml EDTA (Purple-Top) Blood Collection Tube for Plasma**

At the baseline visit, draw blood in the following order:

1. **10 ml Serum (Red-top) Tube for Serum**
2. **10 ml EDTA (Purple-Top) Blood Collection Tube for Buffy Coat and Plasma**

At 6 & 12 month visits, draw the following:

1. **10 ml Serum (Red-top) Tube for Serum**
2. **6 ml EDTA (Purple-Top) Blood Collection tube for Plasma ac-his and PK (X2)**
3. **10 ml EDTA (Purple-Top) Blood Collection tube for Plasma banking (X1)**

SPECIFIC INSTRUCTIONS FOR COLLECTION AND PROCESSING OF EACH SAMPLE ARE DETAILED ON THE FOLLOWING PAGES.

6.1 Labeling Samples

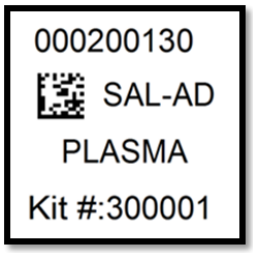
Label Type Summary

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

Each kit is supplied with labels for each specimen to be shipped to NCRAD.

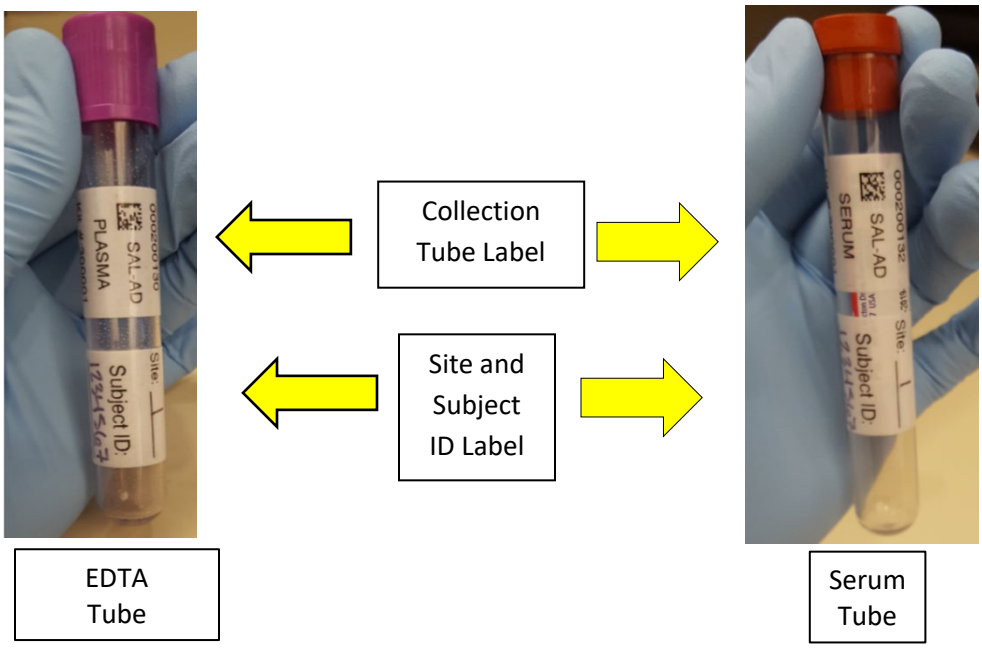
The **Kit Number Labels** do not indicate a specimen type. Place one Kit Number Label within the designated location on the "Biological Sample and Shipment Notification Form" and the "CSF Sample and Shipment Notification Form", if collected. Place the other Kit Number Label on the lid of the corresponding cryovial box. See Section 8.0 for further instructions.

Place one **Site and Subject ID Label** on each blood collection tube (Serum, EDTA). Do not send NCRAD the blue cap conical tubes used to collect CSF or mixed plasma aliquoted from different EDTA tubes. Collection and processing site staff may write on this conical tube for their own reference. The blue cap conical tubes will not have a label. Discard according to your institution's guidelines.



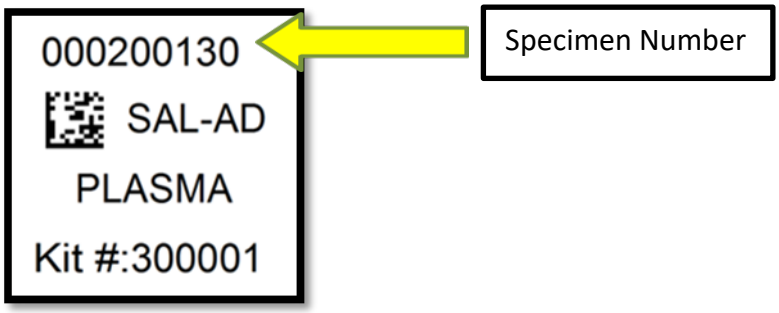
Place the **Collection Tube and Aliquot Label** on the collection tube and/or the aliquot cryovial. Each collection tube will contain two labels: the Site and Subject ID label and the Collection Tube Label. (Pictured below)

The Aliquot Labels intended for the CSF cryovials will contain a different kit number than that of the patient's blood derived specimens from the same visit.

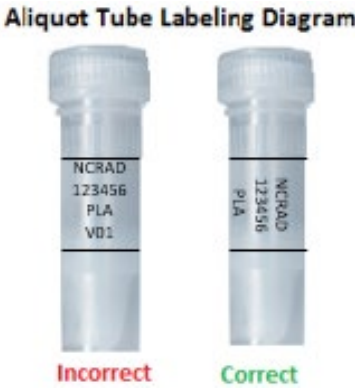


To ensure the label adheres properly and remains on the tube, please follow these instructions:

- Appropriately fill-in Site and Subject ID Labels before adhering to the tube.
- Place blood Collection Tubeand Aliquot Labels on **ALL** collection tubes and aliquot 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.
- Place cryovials in numerical order based on the specimen number, located at the top of the label. This ensures that no aliquot is misplaced or lost during the shipment process (see depiction below).



- Using a fine point sharpie, fill-in and place the Site and Subject ID Labels on the collection tubes only (Serum, EDTA) **BEFORE** sample collection, processing or freezing. These labels are in addition to the Kit Number Labels. **DO NOT** place subject ID labels on any cryovials.
  - The blood Collection Tube and Aliquot Labels contain a 2D barcode on the left hand side of the label. Affix the label so this barcode toward the tube cap.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see labeling diagram below).
- Take a moment to ensure the label is **completely adhered** to the tube. It may be helpful to roll the tube between your fingers after applying the label.



6.2 Video List

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

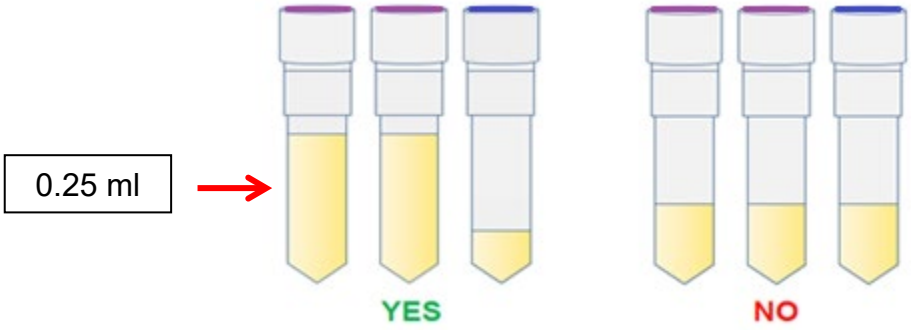
  - Frozen Shipping
  - Plasma and Buffy Coat Processing and Aliquoting
  - Serum Processing and Aliquoting
  - CSF Aliquoting
  - SAL-AD MOP Training

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 aliquot tube should be filled to the assigned volume after processing is completed (refer to detailed processing instructions for average yield per sample).

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

Aliquot the remaining biologic material as the residual volume and ship to NCRAD. Ship *all* material to NCRAD. Fill as many aliquot tubes as possible. For example, if 2.7 ml of a plasma sample is obtained, fill 10 cryovials each with 0.25 ml, and one additional cryovial with the remaining 0.2 ml.



Please note: It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume (anything under 0.25 ml). Please highlight that the aliquot contains a small volume by utilizing the cryovial with a blue sticker on the 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 sticker color and type of aliquot.

Cap Color	Sample Type
Clear Cap with Red Sticker	Serum
Clear Cap with Purple Sticker	Plasma
Clear Cap with Blue Sticker	Residual Aliquot (Plasma, Serum or CSF)
Clear Cap with no sticker	Buffy Coat
Clear Cap with White Sticker	CSF Aliquot

6.4 Screening Visit: 6 ml EDTA (Purple-Top) Blood Collection Tube

Whole Blood Collection for Isolation of Plasma: 6 ml EDTA (Purple-Top) Blood Collection Tubes (for processing of plasma aliquots for ac-his).

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

At the screening visit, draw ONE:

- **6 ml EDTA (Purple-Top) Blood Collection Tube**
- **Only tube drawn at this visit**

1. Place completed Site and Subject ID Label and a Collection Tube label with the specimen type "**PLASMA ac-his**" on the 6 ml EDTA (Purple-Top) Blood Collection Tube. Place similar "**PLASMA ac-his**" aliquot labels on each of the 0.5 ml siliconized cryovials (clear caps, purple stickers).

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

3. Pre-chill centrifuge at 4°C before use.

4. Using a blood collection set and a holder, collect blood into the **6 ml EDTA tubes** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

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

5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 6 ml of blood into each tube.

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

7. Centrifuge the balanced tubes for 15 minutes at 1500 RCF (x g) and 4°C. EDTA Tubes must be spun, aliquoted, and stored upright in a -80°C freezer within 2 hours of the collection time. **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 in your particular rotor).**

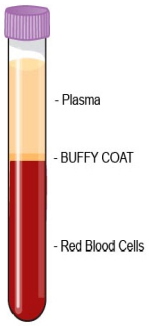
   - Equivalent rpm for spin at 1500 x g
   - While centrifuging, record the time of centrifuge start on the Biological Shipment and Notification Form.

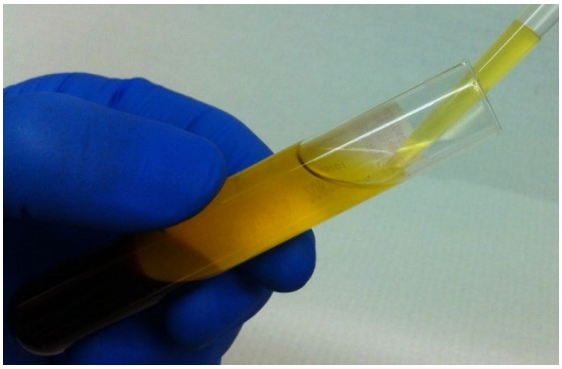
8. Remove the plasma, being careful not to agitate the packed blood cells at the bottom of the collection tube by tilting the tube and placing the pipette tip along the lower side of the wall without touching the red blood cells. This will ensure that the plasma is not contaminated by RBC's (see below).

9. Using the pipetman, transfer plasma into the pre-labeled cryovials. Aliquot 0.25 ml per cryovial. Each 6 ml EDTA tube should yield, on average, 3 ml of blood plasma per tube.

10. For this visit, be sure to only place **plasma** in cryovials labeled **"PLASMA ac-his'.** Take caution not to disturb the blood cells at the bottom of the tube.

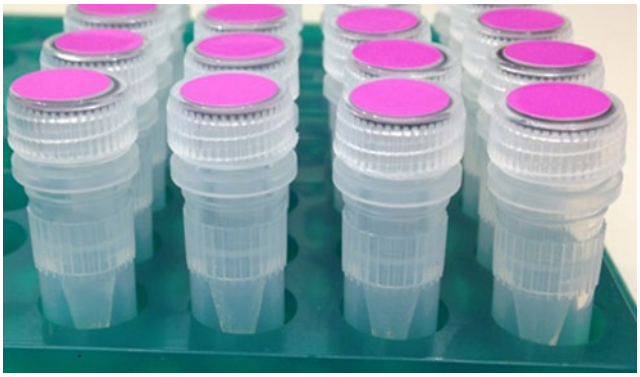
11. Deposit residual plasma (<0.25 ml) in the cryovial with the clear cap and blue sticker to highlight which aliquot contains a smaller volume. Document the specimen number and volume on the available field of the Biological Sample and Shipment Notification Form.





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.

Plasma Aliquots (12 total possible per 6 ml EDTA tube)

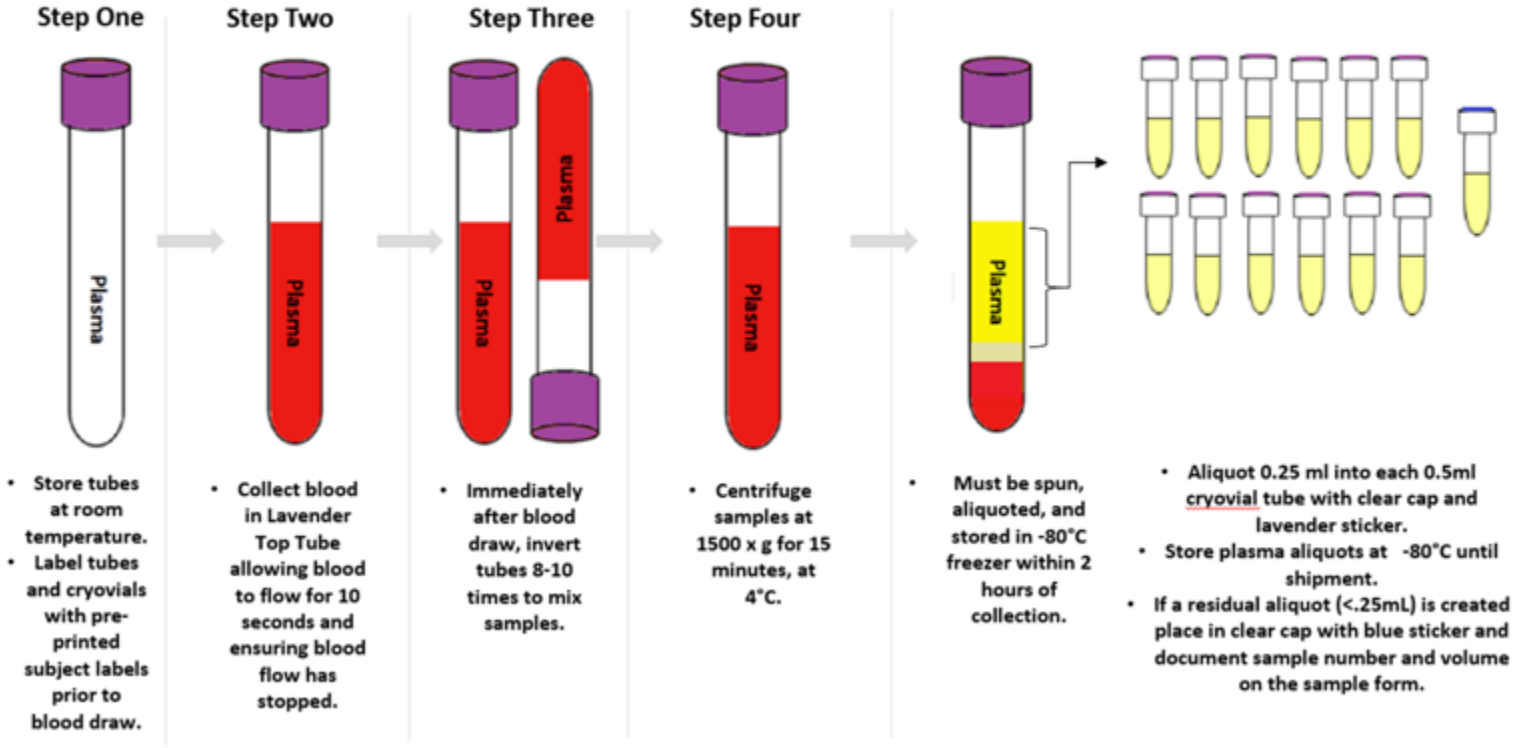


Close up view of 0.5 ml Siliconized Cryovial

12. Place the labeled cryovials upright in one 81 slot cryovial box and place on dry ice. Transfer to **-80°C Freezer as soon as possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice.

13. Dispose of collection with buffy coat and blood cells according to your site's guidelines for disposing of biomedical waste.

Screening Plasma Preparation (6ml Lavender Top Tube)



- ❖ One 6 ml EDTA (Lavender-Top) Blood Collection Tube will yield 3 ml of plasma per tube
  - ❖ 6-12 Plasma ac-his aliquots with 0.25 ml per aliquot

6.5 Baseline Visit: 10 ml Serum (Red-Top) Tube and 10 ml EDTA (Purple-Top) Blood Collection Tube for Plasma

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

At the baseline visit, draw ONE of each tube type:

- **10 ml Serum (Red-Top) Blood Collection Tube**
- **10 ml EDTA (Purple-Top) Blood Collection Tube**

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

Draw Order at the baseline visit:

1. **Draw Serum tube FIRST**
2. **Draw EDTA tube SECOND**

6.5.1. 10 ml Serum (Red-Top) Tube for Serum Collection

Whole Blood Collection for Isolation of Serum: 10 ml Serum Determination (Red-Top) Tube. One Red-Top tube is collected at the Baseline study visit.

1. Place completed Site and Subject ID and pre-printed "SERUM" collection tube label on the red-top serum tube. Place pre-printed "**SERUM**" labels on the (20) 0.5 ml siliconized cryovials that have clear caps with red stickers.

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

3. Pre-chill centrifuge at 4°C before use.

4. Using a blood collection set and a holder, collect blood into**: 10 ml Serum (Red-Top) Tube** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

a. Place donor's arm in a downward position.

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

5. Allow at least 10 seconds for a complete blood draw to take place in the 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 10 ml of blood into the tube.

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

7. **CRITICAL STEP: Allow blood in Serum Tube to clot at room temperature by placing it upright in a vertical position in a tube rack for 30 minutes. Centrifugation must start within 60 minutes of blood draw.**

8. After 30 minutes of clotting, centrifuge the Serum collection tube for 15 minutes at 1500 rcf (x g) at 4°C. Serum samples need to be spun, aliquoted, and stored upright in a -80°C freezer within 2 hours of the time of collection. **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 of any particular rotor. http://www.sciencegateway.org/tools/rotor.htm)**.
   - Equivalent rpm for spin at 1500 x g
   - While centrifuging, record the centrifugation start time on the Biological Sample and Shipment Notification Forms (Appendix B, Appendix C, Appendix D).

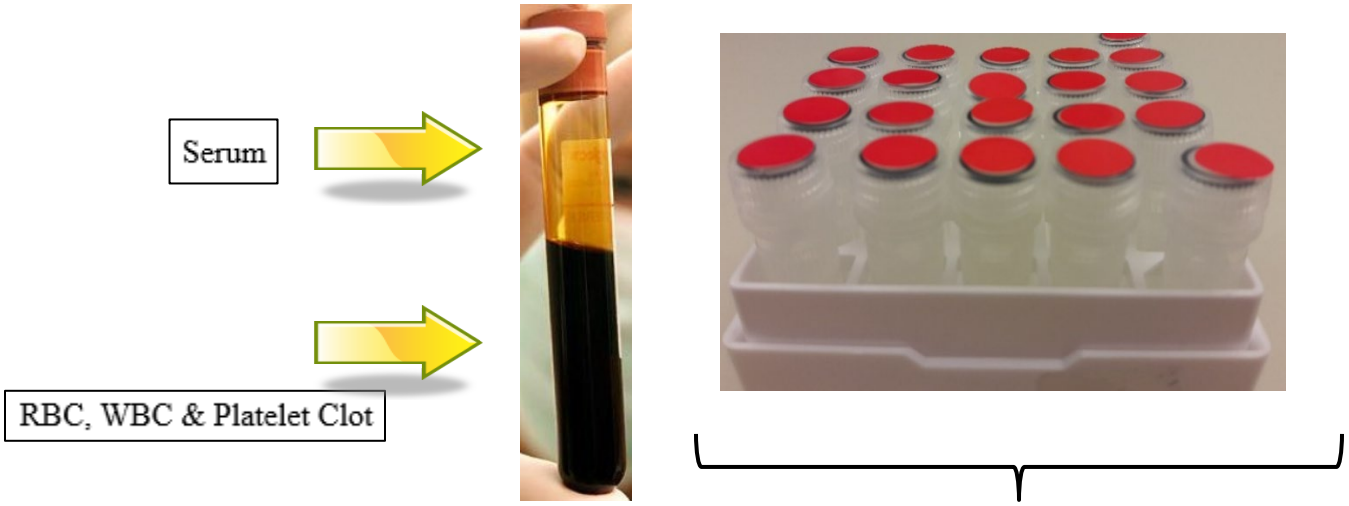
9. Remove the serum, being careful not to disturb the clot at the bottom of the collection tube by tilting the tube and placing the pipette tip along the lower side of the wall without touching the clotted pellet so that serum is not contaminated by red blood cells (pellet material).

10. Using a pipetman, transfer serum into the pre-labeled cryovials. Aliquot 0.25 ml per cryovial. The red-top tube should yield, on average, 5 ml of blood serum per tube.

11. Be sure to only place **serum** in siliconized cryovials that have clear lids and red stickers labeled with the **"SERUM"** label.

12. Deposit residual serum (<0.25 ml) in the cryovial with the clear cap and blue sticker to highlight which aliquot contains a smaller volume. Document the specimen number and volume on the available field of the Biological Sample and Shipment Notification Form.

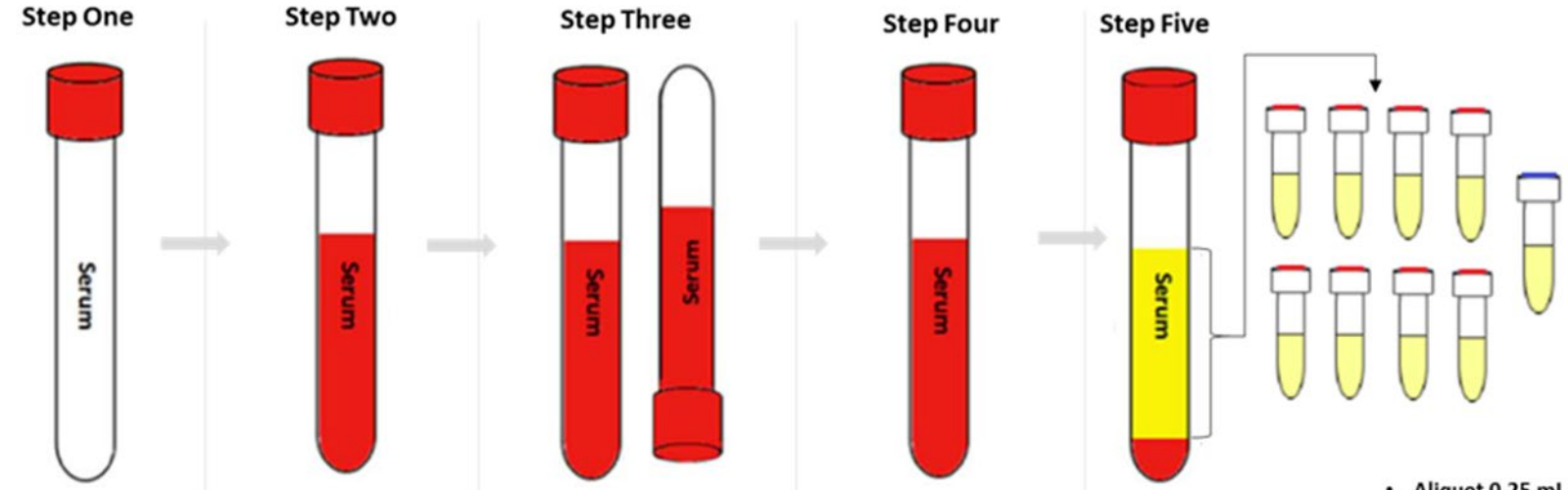
NOTE: When pipetting serum from the serum tube be very careful to pipette the serum top layer only, leaving the clotted cell layer untouched.



Close up view of 0.5 ml Siliconized Cryovial

13. Place the labeled cryovials in the 81-slot cryobox and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice. Dispose of collection tube with pellet in the bottom of the tube according to your site's guidelines for disposing of biomedical waste.

Serum Preparation (10ml Red Top Tube)



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

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

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

- Allow blood to clot for 30 minutes.
- Within 60 minutes of blood draw, centrifuge samples at 1500 x g for 15 minutes at 4°C.

- Must be spun, aliquoted, and stored in -80°C freezer within 2 hours of collection.

- Aliquot 0.25 mL into each 0.5 cryovial tube with the clear cap and red sticker.
- Store plasma aliquots at -80°C until shipment.
- If a residual aliquot (<0.25 mL) is created, use the clear cap with blue sticker and record the sample number and volume on the sample form.

At Baseline, 6 & 12 Month Visits Only

6.5.2. Whole Blood Collection for Isolation of Plasma: 10 ml EDTA (Purple-Top) Blood Collection Tubes (for processing of plasma aliquots and for DNA from buffy coat)

1. Place completed Site and Subject ID and pre-printed "**PLASMA**" collection tube label on the 10 ml EDTA (Purple-Top) Blood Collection Tube. Place pre-printed "**PLASMA**" aliquot labels on the 0.5 ml siliconized cryovial with clear caps and purple stickers.

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

3. Pre-chill centrifuge at 4°C before use.

4. Using a blood collection set and a holder, collect blood into the **10 ml EDTA tubes** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

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

5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into each tube.

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

7. Centrifuge balanced tubes for 15 minutes at 1500 RCF (x g) at 4°C. EDTA Tubes must be spun, aliquoted, and stored upright in a -80°C freezer within 2 hours of the time of collection. **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 in your particular rotor).

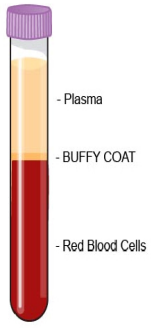
- Equivalent rpm for spin at 1500 x g
- While centrifuging, record the time of centrifuge start on the Biological Shipment and Notification Form.

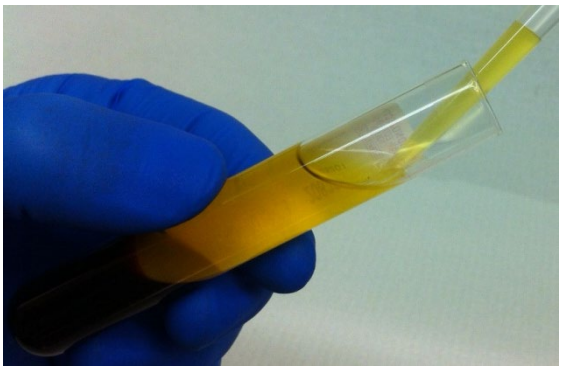
8. Remove the plasma, being careful not to agitate the packed blood cells at the bottom of the collection tube by tilting the tube and placing the pipette tip along the lower side of the wall without touching the pellet. This will ensure the plasma is not contaminated by pellet material (see below).

9. Using a pipetman, transfer plasma into the pre-labeled cryovials. Aliquot 0.25 ml per cryovial.

10. Each EDTA tube should yield, on average, 5 ml of blood plasma per tube. Be sure to only place **plasma** in cryovials labeled with **"PLASMA"** labels**.** Take caution not to disturb the blood cells (cell pellet) at the bottom of the tube.

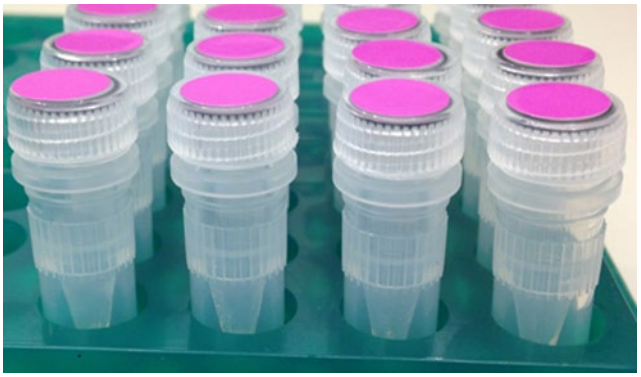
11. Deposit residual plasma (<0.25 ml) in the cryovial with the clear cap and blue sticker to highlight which aliquot contains a smaller volume. Document the specimen number and sample volume on the available field of the Biological Sample and Shipment Notification Form.





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.

Plasma Aliquots (20 cryovials total possible per 10 ml EDTA tube)

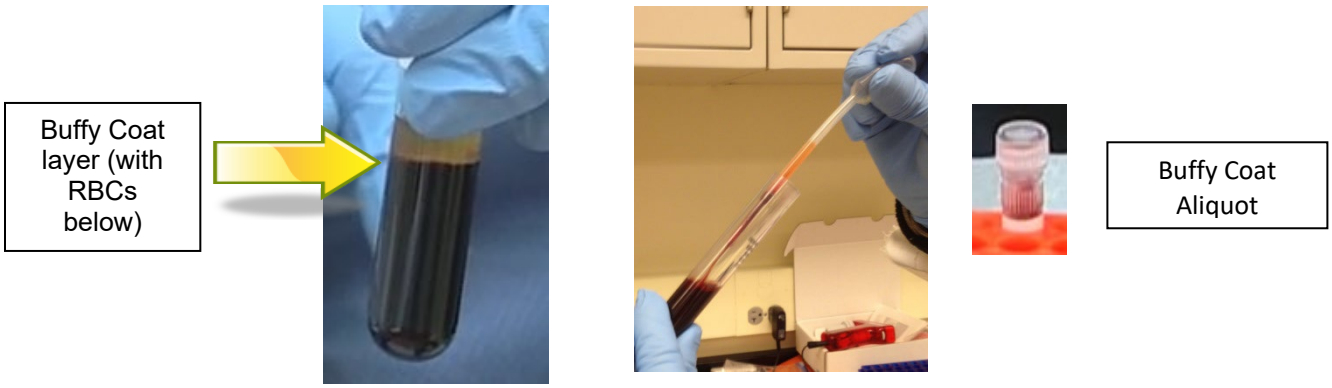


Close up view of 0.5 ml Siliconized Cryovial

12. Store the labeled cryovials in one 81-slot cryovial boxes and place upright on dry ice. Transfer to **-80°C Freezer as soon as possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice.

13. After plasma has been aliquoted from each 10 ml EDTA (Purple-Top) Blood Collection Tube, aliquot the buffy coat layer (see figure) into the labeled 2 ml cryovial with clear cap using pipetman.

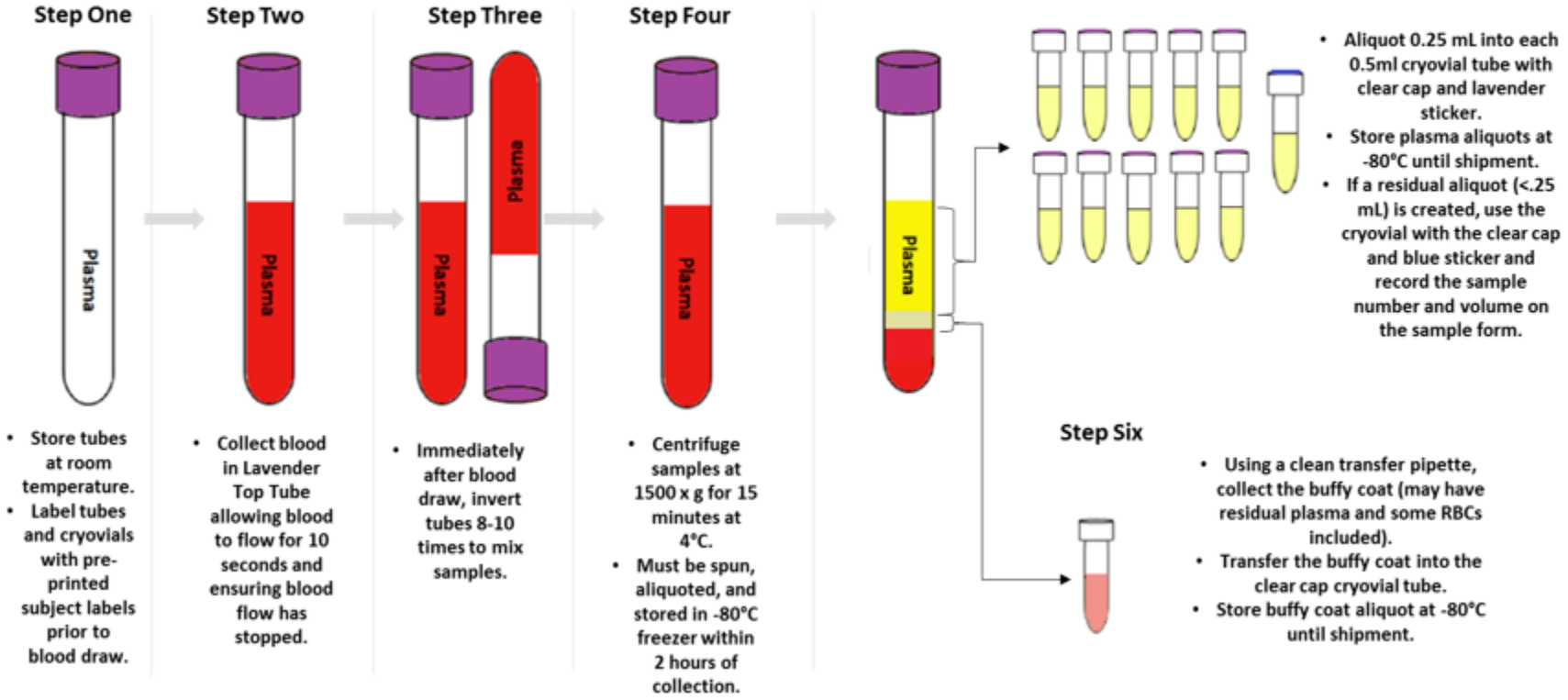
14. The RBC's collected along with the buffy coat will give the aliquot a reddish color. Be sure to place the buffy coat into the cryovial with the clear cap and "BUFFY COAT" label.



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

16. Place the labeled cryovial in the 81-slot cryovial box with the plasma aliquots and place upright on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice.

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



- One 10 ml EDTA (Lavender-Top) Blood Collection Tube will yield approximately:
  - 14-20 Plasma aliquots with 0.25 ml per aliquot for banking
  - 1 Buffy Coat aliquot with 1.0 ml per aliquot for DNA extraction

6.6 6 and 12 month visits: 10 ml Serum (Red-Top) Tube x 1, 6 ml EDTA (Purple-Top) Blood Collection Tube x 2, and 10 ml EDTA (Purple-Top) Blood Collection Tube x 1

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

Draw Order at the baseline visit:

1. **Draw 10 ml Serum tube FIRST (X1)**
2. **Draw 6 ml EDTA tubes SECOND (X2)**
3. **Draw 10 ml EDTA tubes THIRD (x1)**

6.6.1 Serum (Red-Top) Tube (10 ml) for Serum Collection

Whole Blood Collection for Isolation of Serum: 10 ml Serum (Red-Top) Tube. One Red-Top tube is collected at the 6 and 12 month study visits.

1. Place completed Site and Subject ID and pre-printed "SERUM" collection tube label on the red-top serum tube. Place "**SERUM**" labels on the (20) 0.5 ml siliconized cryovials that have clear caps with red stickers.

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

3. Pre-chill centrifuge at 4°C before use.

4. Using a blood collection set and a holder, collect blood into**: 10 ml Serum (Red-Top) Tube** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

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

5. Allow at least 10 seconds for a complete blood draw to take place in the 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 10 ml of blood into the tube.

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

7. **CRITICAL STEP: Allow blood in Serum tube to clot at room temperature by placing it upright in a tube rack for 30 minutes. Centrifugation must start within 60 minutes of blood draw.**

8. After 30 minutes of clotting, centrifuge the Serum collection tube for 15 minutes at 1500 rcf (x g) at 4°C. Serum samples need to be spun, aliquoted, and stored upright in a -80°C freezer within 2 hours of the time of collection. **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 with a particular rotor.**

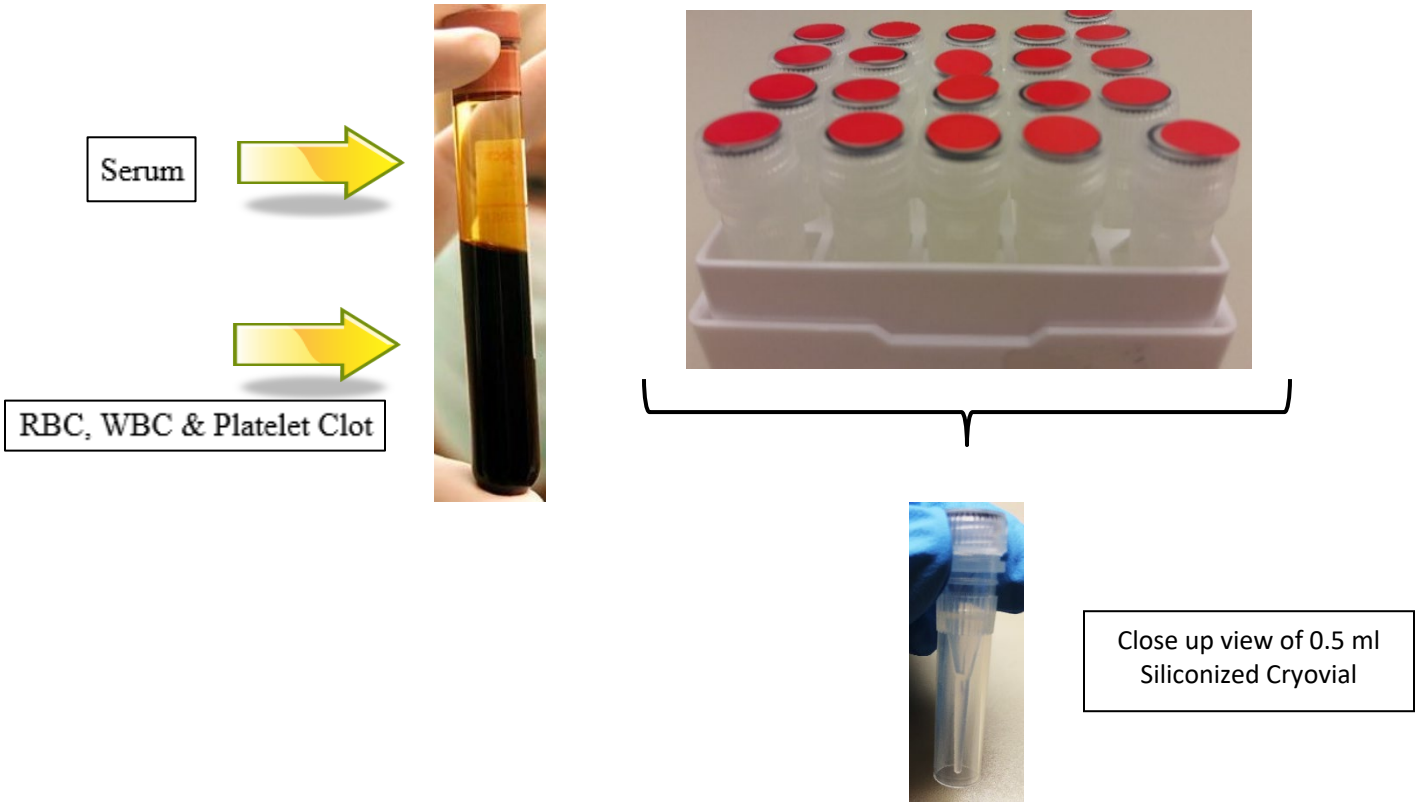
   - Equivalent rpm for spin at 1500 x g
   - While centrifuging, record the centrifugation start time on the Biological Sample and Shipment Notification Forms (Appendix B, Appendix C, Appendix D).

9. Remove the serum, being careful not to disturb the clot at the bottom of the collection tube by tilting the tube and placing the pipetman tip along the lower side of the wall without touching the clotted pellet. This will ensure that serum is not contaminated by pellet material.

10. Using a pipetman, transfer serum into the pre-labeled cryovials. Aliquot 0.25 ml per cryovial. The red-top tube should yield, on average, 5 ml of blood serum per tube. Be sure to only place **serum** in siliconized cryovials that have clear lids with red stickers and are labeled with the **"SERUM"** label.

11. Deposit any residual aliquot (<0.25 ml) in the cryovial with the blue cap to highlight which aliquot contains a smaller volume. Document the specimen number and aliquot volume on available field of the Biological Sample and volume and Shipment Notification Form.

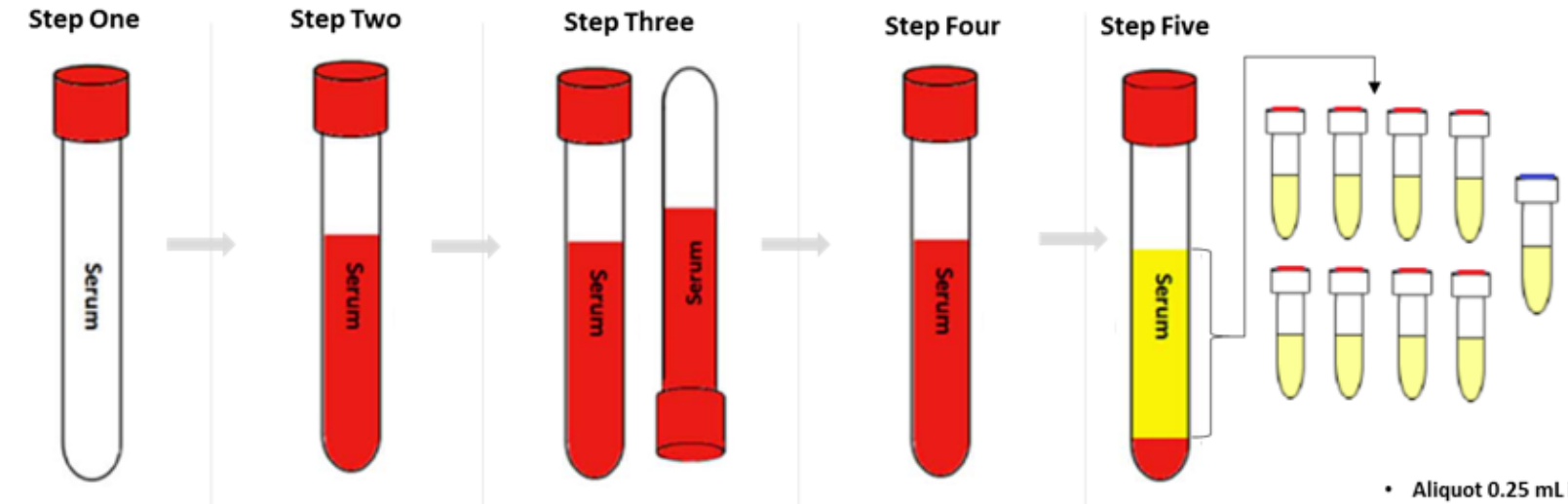
NOTE: When pipetting serum from the serum tube be very careful to pipette the serum top layer only, leaving the clotted cell layer untouched.



12. Place the labeled cryovials in the labeled 81-slot cryobox and place upright on dry ice. Transfer to **-80°C Freezer as soon as possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice.

13. Dispose of collection tube with pellet in the bottom of the tube according to your site's guidelines for disposing of biomedical waste.

Serum Preparation (10ml Red Top Tube)



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

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

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

- Allow blood to clot for 30 minutes.
- Within 60 minutes of blood draw, centrifuge samples at 1500 x g for 15 minutes at 4°C.

- Must be spun, aliquoted, and stored in -80°C freezer within 2 hours of collection.

- Aliquot 0.25 mL into each 0.5 cryovial tube with the clear cap and red sticker.
- Store plasma aliquots at -80°C until shipment.
- If a residual aliquot (<0.25 mL) is created, use the clear cap with blue sticker and record the sample number and volume on the sample form.

At Baseline, 6 & 12 Month Visits Only

6.6.2 Whole Blood Collection for Isolation of Plasma: 6 ml EDTA (Purple-Top) Blood Collection Tube x 2.

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

At the 6 and 12 month visits, draw TWO:

- **10 ml Serum Tube (Red Top) for Serum collection**
- **6 ml EDTA (Purple-Top) Blood Collection Tube for Buffy Coat and Plasma Banking**

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

At the baseline, 6 month, and 12 month visits:

3. **Draw Serum tube FIRST**
4. **Draw EDTA tubes SECOND**

1. Place completed Site and Subject ID and a "**PLASMA PK**" collection tube label on one of the 6 ml EDTA (Purple-Top) Blood Collection Tube and a "**PLASMA ac-his"** collection tube label on the other. Place "**PLASMA PK**" or "**PLASMA ac-his**" aliquot labels on the 0.5 ml siliconized cryovials that have clear caps with purple stickers.

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

3. Pre-chill centrifuge to 4°C before use.

4. Using a blood collection set and a holder, collect blood into the **6 ml EDTA tubes (x2)** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

a. Place donor's arm in a downward position.
b. Hold tube in a vertical position, below the donor's arm during blood collection.
c. Release tourniquet as soon as blood starts to flow into last tube of the sequence.

d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 6 ml of blood into each tube.

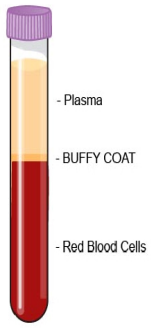
6. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tubes 8 – 10 times.**

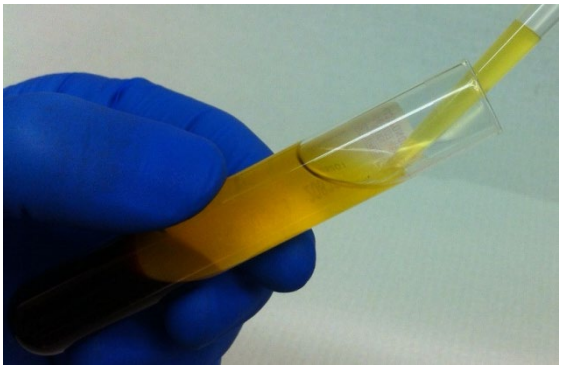
7. Centrifuge the balanced tubes for 15 minutes at 1500 RCF (x g) at 4°C. These EDTA tubes must be spun, aliquoted, and stored upright in a -80°C freezer within 2 hours of the collection time. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM in your particular rotor).**
   - Equivalent rpm for spin at 1500 x g
   - While centrifuging, record the time of centrifuge start on the Biological Shipment and Notification Form.

8. Remove the plasma, being careful not to agitate the packed blood cells at the bottom of the collection tube, by tilting the tube and placing the pipette tip along the lower side of the wall without touching the pellet. This will ensure that the plasma is not contaminated by pellet material (see below).

9. Using a pipetman, transfer plasma into the pre-labeled cryovials for either Plasma PK or Plasma ac-his draw. Aliquot 0.25 ml per cryovial. Each EDTA tube should yield, on average, 3 ml of blood plasma per tube. Be sure to only place **plasma** in cryovials labeled with **"PLASMA PK**" or "**PLASMA ac-his"** labels**.** Take caution not to disturb the blood cells (pellet material) at the bottom of the tube.

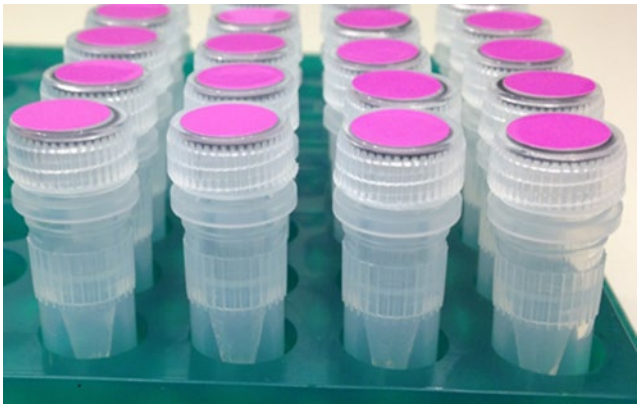
10. Deposit any residual plasma (<0.25 ml) in the cryovial with the clear cap and blue sticker to highlight which aliquot contains a smaller volume. Document the specimen number and aliquot volume on the available field of the Biological Sample and Shipment Notification Form.





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.

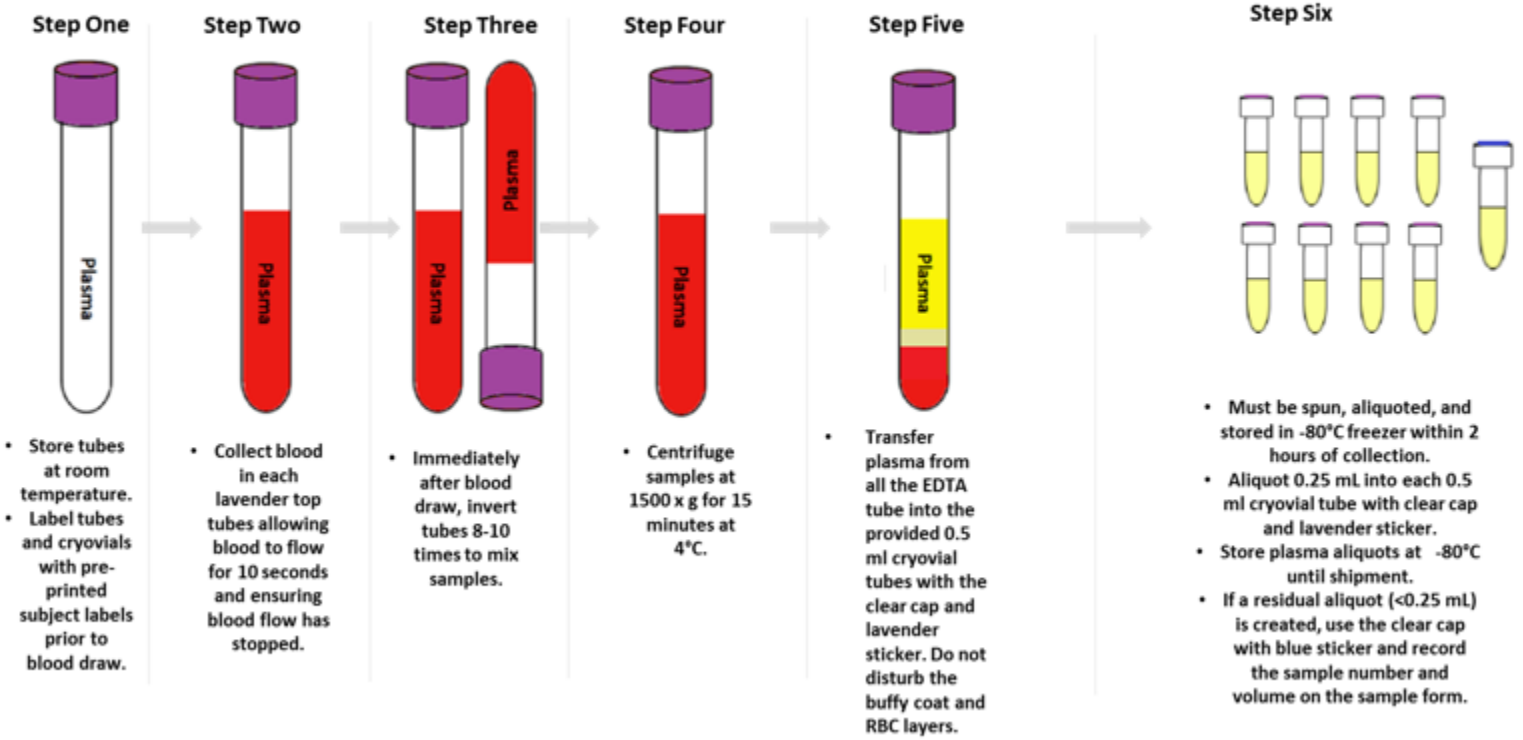
Plasma Aliquots (8-12 possible per tube at 6/12 month visits)



Close up view of 0.5 Siliconized Cryovial

11. Place the labeled cryovials in one 81-slot cryovial box and place upright on dry ice. Transfer to **-80°C Freezer as soon as possible**. Store all samples upright in freezer at **-80°C until shipped** to NCRAD on dry ice.

6 and 12 Month Visits Plasma Preparation (6ml Lavender Top Tube) x2



- **One 6.0 ml EDTA (Lavender-Top) Blood Collection Tube will yield 3 ml of plasma per tube**
  - **6-12 Plasma ac-his aliquots with 0.25 ml per aliquot**
  - **6-12 Plasma PK aliquots with 0.25 ml per aliquot**

6.6.3. Whole Blood Collection for Isolation of Plasma: 10 ml EDTA (Purple-Top) Blood Collection Tubes (for processing of plasma aliquots)

1. Place completed Site and Subject ID and "**PLASMA**" collection tube label on the 10 ml EDTA (Purple-Top) Blood Collection Tube. Place "**PLASMA**" aliquot labels on the 0.5 ml siliconized cryovial with clear caps and purple stickers.

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

3. Pre-chill centrifuge at 4°C before use.

4. Using a blood collection set and a holder, collect blood into the **10 ml EDTA tubes** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

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

5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into each tube.

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

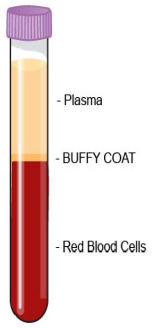
7. Centrifuge balanced tubes for 15 minutes at 1500 RCF (x g) at 4°C. EDTA Tubes must be spun, aliquoted, and stored upright in a -80°C freezer within 2 hours of the time of collection. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM in your particular rotor).**
   - Equivalent rpm for spin at 1500 x g

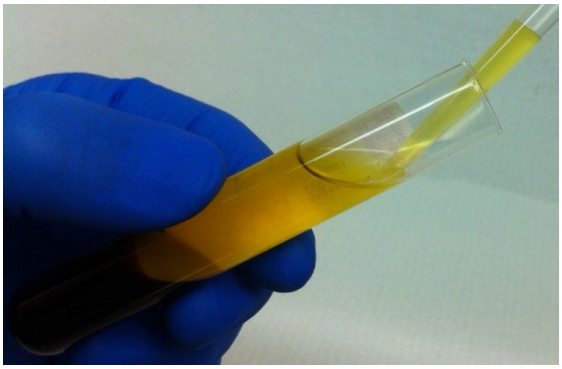
- While centrifuging, record the time of centrifuge start on the Biological Shipment and Notification Form.

8. Remove the plasma, being careful not to agitate the packed blood cells at the bottom of the collection tube by tilting the tube and placing the pipette tip along the lower side of the wall without touching the pellet. This will ensure the plasma is not contaminated by pellet material (see below).

9. Using a pipetman, transfer plasma into the pre-labeled cryovials. Aliquot 0.25 ml per cryovial. Each EDTA tube should yield, on average, 5 ml of blood plasma per tube. Be sure to only place **plasma** in cryovials labeled with **"PLASMA"** labels**.** Take caution not to disturb the blood cells (cell pellet) at the bottom of the tube.

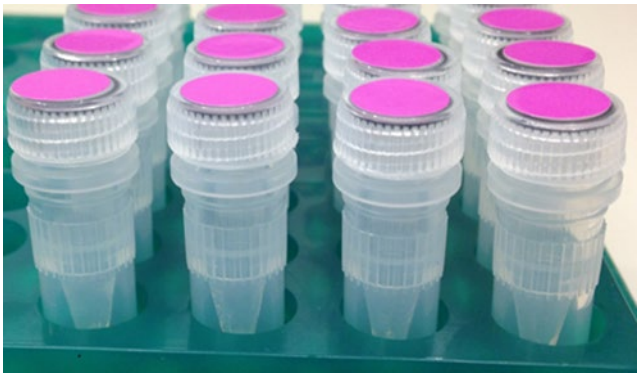
10. Deposit residual plasma (<0.25 ml) in the cryovial with the clear cap and blue sticker to highlight which aliquot contains a smaller volume. Document the specimen number and sample volume on the available field of the Biological Sample and Shipment Notification Form.





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.

Plasma Aliquots (20 cryovials possible per 10ml EDTA tube)



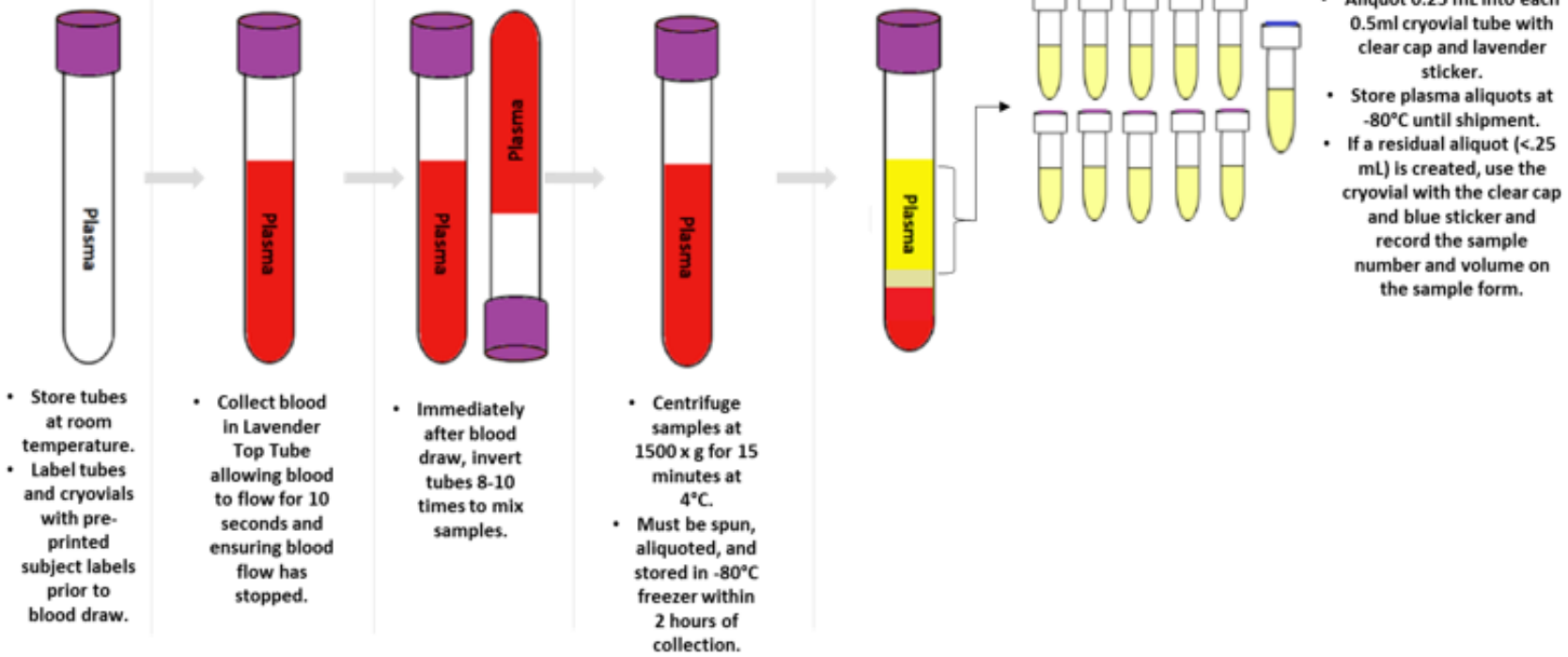
Close up view of 0.5 ml Siliconized Cryovial

11. Store the labeled cryovials in one 81-slot cryovial boxes and place upright on dry ice. Transfer to **-80°C Freezer as soon as possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice.

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

13. Place the labeled cryovial in the 81-slot cryovial box with the plasma aliquots and place upright on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice.

6 & 12 Month Visits Plasma (10ml Lavender Top Tube)



- One 10 ml EDTA (Lavender-Top) Blood Collection Tube will yield approximately:
  - 14-20 Plasma aliquots with 0.25 ml per aliquot for banking

6.7 Sample Redraws

There may be situations where a patient sample needs to be redrawn for certain visits. At those times, NCRAD study staff will alert site coordinators that a participant sample has failed and should be redrawn. This can happen for several reasons, including insufficient blood in initial sample, temperature storage extremes, or even shipping errors.

Tubes that may be redrawn using the redraw kit that includes a 10 ml EDTA (Purple-Top) Blood Collection Tube.

Please note: This 10 ml EDTA (Purple-Top) Blood Collection Tube may be drawn any day of the week. If an EDTA tube is drawn on a Friday for a redraw, it will be used for DNA extraction only. It should not be spun down for plasma isolation, please hold sample at room temperature until it can be shipped the following Monday. Samples drawn on Monday-Thursday can be shipped on the same day as the blood draw.

A sample redraw may occur in one of two ways:

1. The subject travels back to the SAL-AD site, the coordinator redraws blood sample, and ships it ambient back to NCRAD.

OR

2. The site staff sends a blood kit directly to the participant's home for the blood draw to be completed by their local phlebotomist or physician. The ambient sample is then shipped by the participant or physician directly to NCRAD.

Please see Appendix G for Biological Sample and Shipment Forms for participants who are provided blood kits for their local physician.

7.0 CEREBROSPINAL FLUID COLLECTION

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

CSF should be collected in the morning between 8am – 10am, preferably fasted. If fasting is not feasible, the low fat diet should be followed (See Appendix F). Record the time of last meal on Biological Sample and Shipment Forms.

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

Collected at the following visits:

- **Screening**
- **6 month**
- **12 month**

7.1 Lumbar Puncture Supplies

The lumbar puncture tray will be provided by the local SAL-AD site. Be sure to check the expiration date: these reflect the expiration date of the lidocaine.

Supplies for collection and shipment of CSF are sent to sites in a separate kit from Indiana University.

Sterile, individually packaged 50 ml conical tubes are available to sites who are completing the Lumbar Puncture using the gravitational method. Because not all sites are utilizing this method, the sterile conical tubes must be requested separately from the visit kit. They are located within the Individual Supply list of the kit request module (Please see Section 5.2).

7.2 Preparing for the Lumbar Puncture

1. On an over bed table, remove the contents of the LP kit from the outer plastic packaging, leaving the contents wrapped in their sterile drape. Supplies should remain wrapped until the person performing the LP is seated and begins examining the subject.

2. Feel the outside of the LP kit (still wrapped) to determine which end contains the spongy swabs. Turn this end toward the person performing the LP and begin unwrapping the kit.

3. Touch only the outside of the paper wrapper. When you grab an edge to unfold it, touch only the folded under portions of the outside of the wrapper. Also, don't let the outside of the wrapper touch any part of the inside.

4. If you touch any part of the paper wrapper, or if any non-sterile object or outside of the wrapper touches any part of the inside of the wrapper, discard the kit and start over.

5. If you are in doubt as to whether something touched the inside of the paper wrapper, throw the kit away and start over.

7.3 Maintaining the Sterile Field

1. A big part of assisting with the LP is keeping the field sterile. To maintain a sterile field, one must keep other people and objects a reasonable distance away from the procedure area remind all assisting in the LP to be careful around it.

2. If anyone touches the inside of the paper wrapper or any of the kit contents, throw away the kit away and start over.

3. If there is any doubt as to whether or not someone touched the kit, throw it away and start over.

4. You are the monitor for whether the person performing the LP has broken sterility. This usually happens by touching something not sterile with a sterile gloved hand. Feel free to speak up and inform people if need be. Be assertive.

7.4 Tips for Clinicians Performing Lumbar Puncture

**Optimizing patient comfort and minimizing the risk of adverse events.*

1. Talk the patient through the procedure so that there are no surprises.

2. Use of a Sprotte 24g atraumatic spinal needle and careful technique are optimal for reducing post-LP headache risk.
    a. A pencil point spinal needle such as Whitacre 24g, Spinocan 22g or 24g may also be used.

3. Use adequate local anesthesia with a 25g 1/2" needle and inject lidocaine to raise a skin wheal. Then, inject lidocaine using the pattern of a square— first the center, and then to all 4 corners.

4. If the subject is thin, do not insert the deep infiltration needle OR the spinal introducer all the way. Use only about 2/3 of their length (to prevent entering the subarachnoid space with anything other than the 24g pencil point spinal needle).

5. Increasing fluid intake immediately after LP is helpful.

6. Be sure to give post-LP care instructions verbally to the subject (see below).

7.5 Post-LP Care Instructions

- Advise the subject to refrain from exertion (e.g., exercise, housework, gardening, lifting, sexual activity, or any other strenuous activities) for 24 hours after the LP.

- Advise the subject to continue with increased fluid intake.

7.5.1 Mild to Moderate headache after a lumbar puncture

- Mild to Moderate headache following lumbar puncture usually resolves within 3-4 days.

- Treatment of Mild to Moderate headache
    - Limit physical activity as much as possible.
    - Oral fluids and caffeine are helpful. Drinking a can of a caffeinated soft drink such as Mountain Dew (for example) is preferable to coffee, which has some diuretic activity.

- Tylenol should be used for symptomatic relief. If a subject cannot tolerate Tylenol, ibuprofen should be used. Avoid aspirin. If these do not relieve the headache, Tylenol with codeine or an equivalent could be considered.

7.5.2 Severe headache after a lumbar puncture

If the headache becomes severe, posturally sensitive (relieved by supine posture), or is accompanied by nausea, vomiting, tinnitus, and/or visual disturbances, the subject should contact the site study staff for further instruction per standard clinical care.

7.6 Detailed Lumbar Puncture Procedure

* See training video for *CSF Processing and Aliquoting:* https://ncrad.org/resource_salad.html .

Place the "**CSF**" label on the collection and aliquot tubes (per Section 6.1). Please remember, the kit number on the CSF labels will be different than the blood aliquots. Prepare the 40 cryovials provided by NCRAD based on the collection of 20 ml of CSF. Additional cryovials may be necessary; these may be retrieved from the SAL-AD Supplemental kit provided to each site.

1. Place cryovials on wet ice prior to the procedure so they are pre-cooled (See below):

2. Perform lumbar puncture using the atraumatic technique.

3. Collect CSF into syringes or sterile conical tube (if a noticeably bloody tap, discard the first 1-2 ml).

4. Collect 20 ml of CSF and transfer to 50 ml conical polypropylene tubes at room temperature. Mix gently by inverting 3-4 times. Record the time of draw (once collection is complete) on the CSF Sample and Shipment Notification Form.

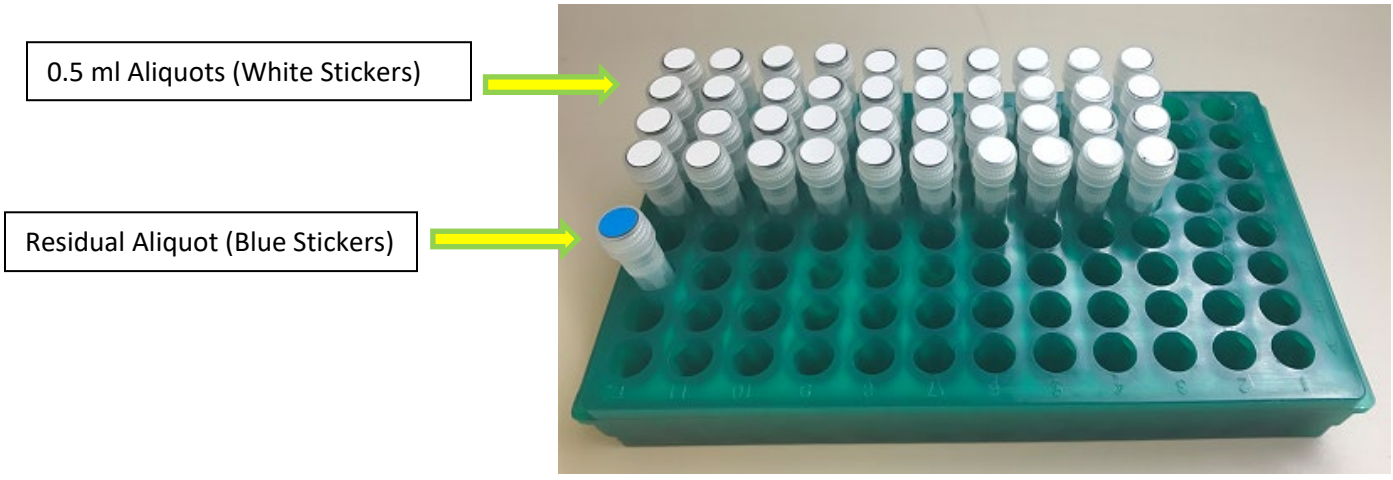
5. Within 15 minutes of collection, spin the remaining CSF sample down at 2000 x g for 10 minutes at room temperature, (64°F-77°F) (18°C- 25°C). For assistance, see Appendix A.

6. Using a clean transfer pipette, transfer all CSF into a second 50ml conical tube, leaving the pellet in the bottom. Mix by inverting the new tube 3-4 times.

7. Pipette (micropipette preferred) 0.5 ml of supernatant directly into pre-cooled polypropylene CSF collection cryovials that have clear caps and white stickers. This will yield, on average, 40 cryovials per subject. (Use more cryovials if needed, do not discard any CSF.)

8. Seal each siliconized cryovial with clear cap and white sticker.

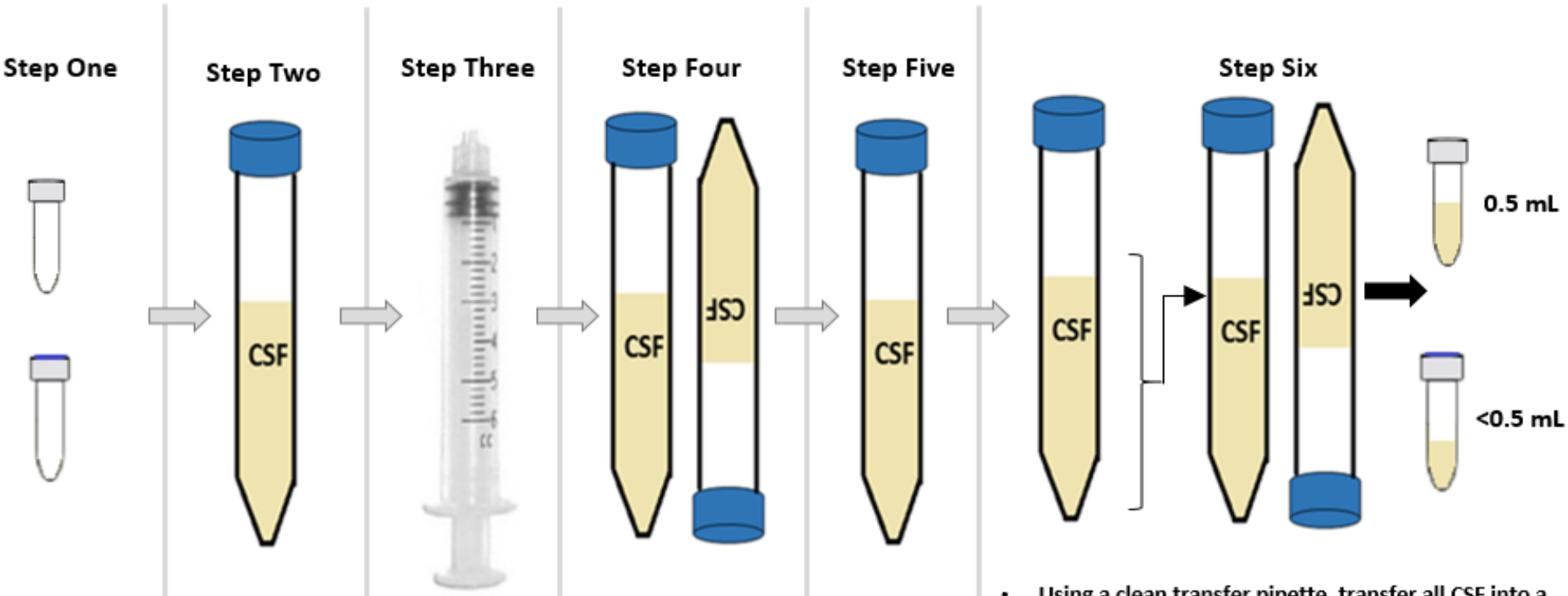
9. Deposit residual CSF (<0.50 ml), in the cryovial with a clear cap and BLUE sticker to indicate that this aliquot has low volume. Record the specimen number and volume on the Biological Sample Shipment Form.



10. Within 60 minutes of CSF collection, freeze aliquots immediately on dry ice and then store upright in a **-80°C freezer until shipment on dry ice** in a provided shipping container is possible.

11. Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the study database.

CSF Preparation



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

- Ensure 50 ml conical tubes are labeled to site specifications.
- Labels will not be provided for these tubes.

- Collect CSF into 6mL luer lock syringe.
- Collection should yield 20 ml.

- Immediately after collection, invert 50 ml conical tube 3-4 times to mix sample.

- Within 15 minutes of collection, centrifuge sample at RT at 2000 x g for 10 minutes.

- Using a clean transfer pipette, transfer all CSF into a second 50mL conical tube, leaving the pellet in the bottom, and mix the new tube gently by inverting 3-4 times
- Aliquot 0.5 ml into cryovials with a clear cap and white sticker
- Aliquot any residual volume into a cryovial with a clear cap and blue sticker
- Document barcode number of residual aliquot on sample form
- Store CSF aliquots at -80° until shipment

8.0 PACKAGING & SHIPPING INSTRUCTIONS

ALL study personnel responsible for shipping should be certified in biospecimen shipping.

Sample Type	SAL-AD Study Visit	Aliquot Volumes	Number sent to NCRAD	Ship
Whole blood (Purple-Top EDTA) for isolation of plasma & buffy coat (for DNA extraction)	**All Visits**	0.25 ml plasma aliquots per 0.5 ml siliconized cryovial (purple stickers)	8-12 cryovials (Screening) 16-20 cryovials (Baseline) 32-44 cryovials (6 & 12 month visits)	Frozen
	Baseline Visit	1 ml buffy coat aliquot per 2 ml clear cap cryovial	1 cryovial (Baseline)	Frozen
Whole blood (Red-Top Serum) for isolation of serum	**Baseline, 6 month, and 12 month visits**	0.25 ml serum aliquots per 0.5 ml siliconized cryovial (red stickers)	16-20 cryovials	Frozen
CSF	**Screening, 6 month, and 12 month visits**	0.5 ml CSF aliquots per 2 ml siliconized cryovial (white stickers)	Up to 40 cryovials	Frozen

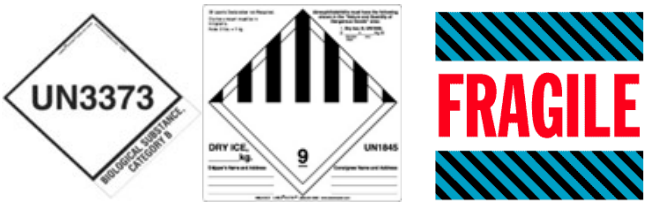
8.1 Frozen Shipping

IMPORTANT!
FROZEN SAMPLES <u>MUST</u> BE SHIPPED MONDAY-WEDNESDAY ONLY!

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

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

- The primary receptacle (frozen cryovials) must be leak proof and in total must not contain more than 1L .
- The secondary packaging (plastic canister or 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) 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 included in shipment must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible Person
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373
  - ✓ Class 9 label including UN 1845, and net weight of dry ice contained



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

8.1.1 NCRAD Packaging and Shipment Instructions – Frozen Shipments

1. Contact FedEx 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:

- Completed Biological Sample and Shipment Notification Form to the email notification.
  (See Appendix B, Appendix C, Appendix D, and/or Appendix E for the NCRAD sample forms)

- If email is unavailable please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment.

3. Place all frozen, labeled 0.25 ml aliquots of plasma and serum, and 0.5 ml aliquots of CSF in the appropriate cryobox (see below).

    i. Each large cryobox holds up to 81 cryovials.
        1. Screening Visit will have a maximum of: 52 aliquots; 12 plasma, 40 CSF.
            a. Use large 81 slot cryobox and place all aliquots for subject in single box.
        2. Baseline Visit will have a maximum of: 41 aliquots; 20 plasma, 1 buffy coat, 20 serum.
            a. Use large 81 slot cryobox and place all aliquots for subject in single box.
        3. 6 and 12 Month Visits will each have a maximum of: 104 aliquots; 12 plasma PK, 12 Plasma ac-his, 20 plasma, 20 serum, 40 CSF.
            a. Use large 81 slot cryobox for blood draw
            b. Use second 81 slot cryobox if CSF collected

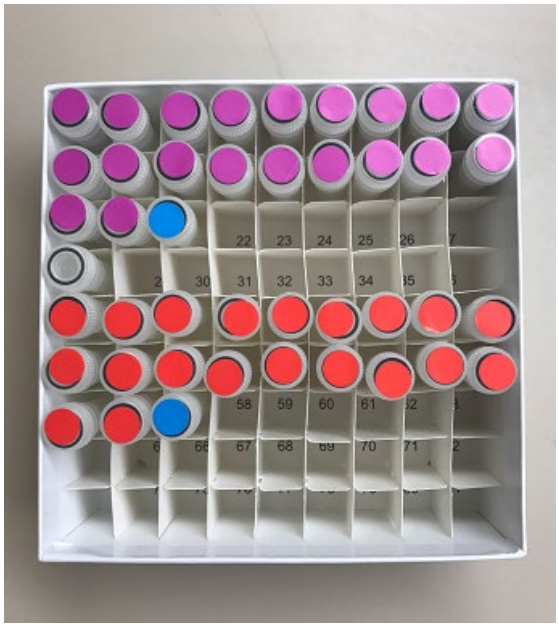
    ii. A cryobox should contain all of the specimens from the same patient, per time point and should have a lid labeled with the proper Kit Number Label/s.

    iii. **Batch shipping should be performed every 3 months or when specimens from 4 participants accumulate, whichever is sooner.**

81-Slot Cryobox Containing Biospecimes from Screening and Baseline Visits

Screening Visit

Baseline Visit



81-Slot Cryobox Containing Biospecimes from 6 Months and 12 Month Visits

Blood Kit Aliquots:

- Plasma PK
- Plasma ac-his
- Plasma for banking
- Serum

CSF Kit Aliquots:

- CSF

4. Label the lid of each cryobox with the corresponding kit number label(s). Please place the each cryobox in a biohazard bag and seal.
    i. Place large cryoboxes in large Biohazard bags

5. As the cryoboxes are placed in the clear plastic biohazard bags, 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 the lid of each cardboard cryobox prior to inserting into the biohazard bag.

Cryobox placed in clear biohazard bag

6. Place approximately 2-3 inches of 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 dry ice. Ensure that cryoboxes are placed so the cryovials are upright in the shipping container.

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

9. The inner Styrofoam shipping container must contain approximately 45 lbs (or 20kg) of dry ice. The dry ice should entirely fill the inner box and be placed on top of the containers to ensure the frozen state of the specimens.

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 subject visit kit. Close and seal the outer cardboard shipping carton with packing tape.

11. Complete the FedEx return airbill with the following information:
    a. Section 1: From
        i. Fill in your name, address, phone number, and Site FedEx Account Number.
    b. Section 2: Your Internal Billing Reference
        i. Add any additional information required by your site.
    c. Section 6: Special Handling and Delivery Signature Options
        i. Under "Does this shipment contain dangerous goods?" check the boxes for "Yes, Shipper's Declaration not required" and "Dry Ice". Enter the number of packages (1) and the net weight of dry ice in kg.

12. Complete the Class 9 UN 1845 Dry Ice label (B&W diamond) with the following information:
    a. Your name and return address
    b. Net weight of dry ice in kg (must match amount on the airbill)
    c. Consignee name and address:

    SAL-AD at NCRAD
    Indiana University School of Medicine
    351 W. 10th St TK-217
    Indianapolis, IN 46202
    Phone: 1-800-526-2839

d. Do not cover any part of this label with other stickers, including pre-printed address labels.

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

IMPORTANT!
Complete the required fields on the FedEx return airbill and Class 9 Dry Ice label, or FedEx may reject or return your package.

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

15. Specimens should be sent to the address below via FedEx Priority Overnight. Frozen shipments should be sent Monday through Wednesday to avoid shipping delays on Thursday or Friday. FedEx does not replenish dry ice if shipments are delayed or held over during the weekend.

SAL-AD at NCRAD
Indiana University School of Medicine
351 W. 10th St TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

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

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

For frozen shipments, include no more than four subjects worth of cryovial boxes in order to have room for a sufficient amount of dry ice to keep samples frozen up to 24 hours.

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

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

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

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

9.0 Data Queries and Reconciliation

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

The SAL-AD data collection team 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 SAL-AD 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 Biological Sample and Shipment Notification Form and logged at NCRAD compared to information entered into the SAL-AD study 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

Appendix A

Rate of Centrifuge Worksheet

Please complete and return this form by fax or email to the NCRAD Project Manager if you have any questions regarding sample processing. The correct RPM will be sent back to you. Make note of this in your SAL-AD Biologics Manual.

Submitter Information

Name: **Site:**
Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

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

Calculating RPM from G-Force:

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

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

Comments:

Please send this form to NCRAD Study Coordinator

317-321-2003 (Fax) **alzstudy@iu.edu**

Appendix B
Biological Sample and Shipment Notification Form – Screening Visit

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

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

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

From: _____ Site: _____

Phone: _____ Fax: _____

Email: _____ Date: _____

Study: SAL-AD **Kit #:**

Site ID: _____ **Individual ID:** _____

KIT BARCODE

Visit: ***SCREENING***

Sex: ☐M ☐F **Year of Birth:** _____ **CSF Sample Donated?** ☐Yes ☐No

Blood Collection:

1. Date Drawn: _____	2. Time of Draw: 24 hour clock: _____[HHMM]
3. Last time subject ate: Date: _____	4. Last time subject ate: 24 hour clock: _____[HHMM]

Blood Processing:

Plasma (EDTA/Purple Top Tube)	
Time spin started: 24 hour clock:	_____[HHMM]
Duration of centrifuge:	_____minutes
Temp of centrifuge: _____°C	Rate of centrifuge: _____ x g
Original volume drawn (1x 6 mL EDTA tube):	_____ mL
Time aliquoted:	_____[HHMM]
Number of 0.25 mL plasma aliquots created (up to 12): **(Siliconized cryovial: Clear Cap with Purple Sticker):**	_____ x 0.25 mL
If applicable, volume of residual plasma aliquot (less than 0.25 mL): **(Siliconized cryovial: Clear Cap with Purple Sticker):**	_____ mL
If applicable, specimen barcode number of residual: **(Last four digits)**	_____
Time aliquots placed in freezer (24 hour clock):	_____[HHMM]
Storage temperature of freezer:	_____ºC

Notes:_____

Appendix C

Biological Sample and Shipment Notification Form – Baseline Visit

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

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

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

From: _____ Site: _____

Phone: _____ Fax: _____

Email: _____ Date: _____

Study: SAL-AD **Kit #:**

KIT BARCODE

Site ID: _____ **Individual ID:** _____

Visit: ***BASELINE***

Sex: ☐M ☐F **Year of Birth:** _____

Blood Collection:

1. Date Drawn: _____	2. Time of Draw: 24 hour clock: _____[HHMM]
3. Last time subject ate: Date: _____	4. Last time subject ate: 24 hour clock: _____[HHMM]

Blood Processing:

Plasma (EDTA/Purple Top Tube)		**Serum (Serum Separator/Red Top Tube)**	
Time spin started: 24 hour clock:	_____[HHMM]	Time spin started: 24 hour clock: (30 minutes after draw time):	_____[HHMM]
Duration of centrifuge:	_____minutes	Duration of centrifuge:	_____minutes
Temp of centrifuge: _____°C Rate of centrifuge: _____ x g		Temp of centrifuge: ____°C Rate of centrifuge: _____ x g	
Original volume drawn (1x10 mL EDTA tube):	_____ mL	Original volume drawn (1x10 mL Serum tube):	_____ mL
Time aliquoted: 24 hour clock:	_____[HHMM]	Time aliquoted: 24 hour clock:	_____[HHMM]
Number of 0.25 mL plasma aliquots created (up to 20): **(Siliconized cryovial: Clear Cap with Purple Sticker):**	_____ x 0.25 mL	Number of 0.25 mL serum aliquots created (up to 20): **(Siliconized cryovial: Clear Cap with Red Sticker):**	_____ x 0.25 mL
If applicable, **volume of residual** plasma aliquot (less than 0.25 mL): **(Siliconized cryovial: Clear Cap with Blue Sticker):**	_____ mL	If applicable, **volume of residual** serum aliquot (less than 0.25 mL): **(Siliconized cryovial: Clear Cap with Blue Sticker):**	_____ mL
If applicable, specimen barcode number of residual: **(Last four digits)**	_ ____	If applicable, specimen barcode number of residual: **(Last four digits)**	_____
Time aliquots placed in freezer (24 hour clock):	_____[HHMM]	Time aliquots placed in freezer (24 hour clock):	_____[HHMM]
Storage temperature of freezer:	_____ºC	Storage temperature of freezer:	_____ºC
Volume of buffy coat aliquot (one per EDTA tube):	_____mL		

Notes: _____

Appendix D
Biological Sample and Shipment Notification Form – 6 Month/12 Month Visit

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

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

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

From: _____ Site: _____

Phone: _____ Fax: _____

Email: _____ Date: _____

Study: SAL-AD **Kit #:**

Site ID: _____ **Individual ID:** _____

KIT BARCODE

Visit: (Circle One): ***6 MONTH VISIT 12 MONTH VISIT***

Sex: ☐M ☐F **Year of Birth:** _____ **CSF Sample Donated?** ☐Yes ☐No

Blood Collection:

1. Date Drawn: _____	2. Time of Draw: 24 hour clock: _____[HHMM]
3. Last time subject ate: Date: _____	4. Last time subject ate: 24 hour clock: _____[HHMM]

Blood Processing:

Serum (Serum Separator/Red Top Tube)	
Time spin started: 24 hour clock: (30 minutes after draw time):	_____[HHMM]
Duration of centrifuge:	_____minutes
Temp of centrifuge: ____°C	Rate of centrifuge: _____ x g
Original volume drawn (1 x 10 mL Serum tube):	_____ mL
Time aliquoted:	_____[HHMM]
Number of 0.25 mL serum aliquots created (up to 20): **(Siliconized cryovial: Clear Cap with Red Sticker):**	_____ x 0.25 mL
If applicable, volume of residual serum aliquot (less than 0.25 mL): **(Siliconized cryovial: Clear Cap with Blue Sticker):**	_____ mL
If applicable, specimen barcode number of residual: **(Last four digits)**	_____
Time aliquots placed in freezer (24 hour clock):	_____[HHMM]
Storage temperature of freezer:	_____ºC

Notes:

Plasma - PK	
Time spin started: 24 hour clock:	_____[HHMM]
Duration of centrifuge:	_____minutes
Temp of centrifuge: _____°C	Rate of centrifuge: _____ x g
Original volume drawn (1 x 6 mL EDTA tube):	_____ mL
Time aliquoted:	_____[HHMM]
Number of 0.25 mL plasma aliquots created (up to 12): **(Siliconized cryovial: Clear Cap with Purple Sticker):**	_____ x 0.25 mL
If applicable, volume of residual plasma aliquot (less than 0.25 mL): **(Siliconized cryovial: Clear Cap with Blue Sticker):**	_____ mL
If applicable, specimen barcode number of residual: **(Last four digits)**	_____
Time aliquots placed in freezer (24 hour clock):	_____[HHMM]
Storage temperature of freezer:	_____ºC
Plasma – AC-HIS	
Time spin started: 24 hour clock:	_____[HHMM]
Duration of centrifuge:	_____minutes
Temp of centrifuge: _____°C	Rate of centrifuge: _____ x g
Original volume drawn (1x 6 mL EDTA tube):	_____ mL
Time aliquoted:	_____[HHMM]
Number of 0.25 mL plasma aliquots created (up to 12): **(Siliconized cryovial: Clear Cap with Purple Sticker):**	_____ x 0.25 mL
If applicable, volume of residual plasma aliquot (less than 0.25 mL): **(Siliconized cryovial: Clear Cap with Blue Sticker):**	_____ mL
If applicable, specimen barcode number of residual: **(Last four digits)**	_____
Time aliquots placed in freezer (24 hour clock):	_____[HHMM]
Storage temperature of freezer:	_____ºC
Plasma - Banking	
Time spin started: 24 hour clock:	_____[HHMM]
Duration of centrifuge:	_____minutes
Temp of centrifuge: ____°C	Rate of centrifuge: _____ x g
Original volume drawn (1 x 10 mL EDTA tube):	_____ mL
Time aliquoted:	_____[HHMM]
Number of 0.25 mL plasma aliquots created (up to 20): **(Clear cap with Purple sticker Siliconized cryovial):**	_____ x 0.25 mL
If applicable, volume of residual plasma aliquot (less than 0.25 mL): **(Siliconized cryovial: Clear Cap with Blue Sticker):**	_____ mL
If applicable, specimen barcode number of residual: **(Last four digits)**	_____
Time aliquots placed in freezer (24 hour clock):	_____[HHMM]
Storage temperature of freezer:	_____ºC

Appendix E
CSF Sample and Shipment Notification Form

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

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

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

From: _____ Site: _____

Phone: _____ Fax: _____

Email: _____ Date: _____

Study: SAL-AD **Kit #:**

Site ID: _____ **Individual ID:** _____

KIT BARCODE

Visit: (circle one) ***SCREENING*** ***6 MONTH VISIT*** ***12 MONTH VISIT***

Sex: ☐M ☐F **Year of Birth:** _____

CSF Collection:

1. Date of Collection: _____	2. Time of Collection: 24 hour clock: _____[HHMM]
3. Last time subject ate: Date: _____	4. Last time subject ate: 24 hour clock: _____[HHMM]
5. Collection process: ☐ Gravitational **OR** ☐ Pull	

CSF Processing:

Time spin started: 24 hour clock:	_____[HHMM]
Duration of centrifuge:	_____minutes
Temp of centrifuge: _____°C	Rate of centrifuge: _____ x g
Total amount of CSF collected (mL):	_____mL
Time aliquoted:	_____[HHMM]
Number of 0.5mL aliquots created (up to 40 total): (Clear Cap Cryovial with White Sticker):	_____ x 0.5 mL
If applicable, volume of CSF residual aliquot (less than 0.5 mL): (Clear cap with blue sticker cryovial):	_____ mL
If applicable, specimen barcode number of residual: (Last four digits):	_____
Time frozen:	_____[HHMM]

Notes: _____

Appendix F
Low Fat Diet Menu Suggestions

Foods to avoid prior to blood collection:

Avoid: *All Fats and nuts such as:*

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

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

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

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

All red meats or meats containing fat such as pork

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

Appendix G

Purple Top-EDTA Redraw/Take Home Sample Form

TO BLOOD DRAWING PERSONNEL

Samples are housed at Indiana University School of Medicine. It will need to be shipped to the address below. Please use the enclosed pre-addressed FedEx Clinical Pak.

SAL-AD at NCRAD
Indiana University School of Medicine
351 W. 10th St TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

The kit provided contains a collection tube with which to obtain blood from the individual for research purposes. Each kit contains 1 Purple-Top tube and all necessary shipping supplies.

DO NOT REFRIGERATE; STORE AT ROOM TEMPERATURE.
DO NOT DRAW OR SHIP ON FRIDAY OR SATURDAY.
PLEASE SHIP SAME DAY AS BLOOD IS DRAWN.

Instructions for drawing and shipping blood samples:

1. Fill **PURPLE TUBE** completely, if possible.
2. Invert (do not shake) tube eight to ten times after drawing blood to thoroughly mix additive with sample.
3. **Enclose this form in shipment with sample**.
4. Ship samples by **Federal Express** immediately after drawing. Use the enclosed, pre-paid Federal Express mailer. There will be no cost to you or the patient for the shipping.

KIT NUMBER (RECORDED ON LABEL): _____

SUBJECT IDENTIFICATION NUMBER (RECORDED ON LABEL):_____

STUDY SITE ID (RECORDED ON LABEL): _____

DATE BLOOD WAS DRAWN: _____

DONOR YEAR OF BIRTH: _____ **DONOR SEX: _____**