iLEADS Manual of Procedures Update:

V3.2025

Section	Change

International Longitudinal Early-Onset Alzheimer's Disease Study

in collaboration with the

National Centralized Repository for Alzheimer's Disease and Related Dementias

National Centralized Repository for Alzheimer's Disease and Related Dementias

Biospecimen Collection, Processing, and Shipment Manual of Procedures

Version 3.2025

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1.0 Abbreviations

AD	Alzheimer's Disease
ATRI	Alzheimer's Therapeutic Research Institute
BL	Baseline visit
CI	Cognitively Impaired
CLIA	Clinical Laboratory Improvement Amendments
CN	Cognitively Normal
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDC	Electronic Data Capture
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
LP	Lumbar Puncture
LRS	Long Read Sequencing
NaHep	Sodium Heparin
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PBMC	Peripheral Blood Mononuclear Cell
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RNA	Ribonucleic Acid
RPM	Revolutions Per Minute

2.0 Purpose

The collection of biofluids is an important part of the International Longitudinal Early-Onset Alzheimer's Disease Study (iLEADS). 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 iLEADS study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

The following samples will be sent to NCRAD:

- Serum
- Plasma
- Buffy Coat (DNA Extraction)
- Whole Blood
- RNA
- PBMC (optional and stored locally if collected)
- CSF (optional)

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 being provided to NCRAD for the iLEADS 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

Abigail Erickson, BS, CCRP, Study Coordinator
Phone: 317-278-1133
Email: agericks@iu.edu

General NCRAD Contact Information
Phone: 1-800-526-2839 or 317-278-8413
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: www.ncrad.org
iLEADS Study Specific Webpage: https://ncrad.org/resource_leads.html

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

3.2 NCRAD Hours of Operation

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

Frozen samples must be delivered to NCRAD **Monday-Friday only**.

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

3.3 NCRAD Holiday Observations

- 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.

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 – December 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: https://ncrad.org/contact/holiday-closures for additional information.

4.0 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
- Dry ice pellets

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

- Centrifuge capable of ≥ 2000 x g with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:
Dry ice pellets (about approximately 30-45 lbs per shipment)
* if using World Courier, they will provide

5.0 Biofluid Collection

5.1 iLEADS Collection Schedule:

	CI Baseline	CN Baseline	CI Month 12	CN Month 12*	CI Month 24	CN Month 24	CI Month 36	CI Month 48 /Annual visit
Serum	X	X	X	X	X	X	X	X
Plasma	X	X	X	X	X	X	X	X
DNA	X	X	X	X	X	X	X	X
Whole blood for CLIA lab testing	X							
Whole blood for long read sequencing	X	X	*Collected only once over the entire course of a participant's participation in the iLEADS Study. May be collected at longitudinal visits **if not** collected at Baseline*					
RNA	X	X	X	X	X	X	X	X
PBMC **optional*	X	X	X	X	X	X	X	X
CSF **optional*	X	X	X		X	X	X	

*CN M12 CSF may be collected if CSF was not collected at Baseline.

Whole blood is collected in up to six different types of tubes (10 ml plain red-top serum tube, 10ml lavender-top EDTA tubes, 6ml lavender-top EDTA tube, 3ml lavender-top EDTA tube, 2.5 ml PAXgene™ tube, and 10ml green-top Sodium Heparin (NaHep) tubes) for shipment to NCRAD or storage at the local site. The plain red-top serum, 10ml EDTA, and 3ml EDTA tubes are processed locally into serum, plasma, buffy coat, and whole blood fractions; they are then aliquoted, frozen at the study site, and shipped to NCRAD. The 6ml lavender top EDTA tube is frozen locally without further processing. The PAXgene™ tube is frozen locally without further processing. Whole blood collection into Sodium Heparin tubes for PBMCs is optional for iLEADS sites. If collected, PBMC samples are processed and kept locally, and are not 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 10. Guidelines for the processing, storage location, and timing of sample collection are listed in the following tables.

5.2 Biofluid Collection Charts

5.2.1 *Biofluid Collection for CI Participants: Baseline, 12-Months, 24-Months, 36-Months, and 48/Annual visits.*

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
Whole blood for isolation of serum	**Plain Red-Top Serum Blood Collection Tube (10 ml)**	**1**	**N/A**	**N/A**	**N/A**
	SERUM: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	4	1.5 ml serum aliquot per 2.0 ml cryovial (red cap)	Up to 4	Frozen
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	**3**	**N/A**	**N/A**	**N/A**
	PLASMA: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	10	1.5 ml plasma aliquot per 2.0 ml cryovial (lavender cap)	Up to 10	Frozen
	BUFFY COAT: 2.0 ml cryovial	3	1 ml buffy coat aliquot per 2.0 ml cryovial (clear cap)	3	Frozen
Whole blood for testing at the CLIA laboratory *(*when applicable*)*	**EDTA (Lavender-Top) Blood Collection tube (6ml)**	1	N/A	1	Frozen
Whole blood for long read sequencing *(*collected only once)*	**EDTA (Lavender-Top) Blood Collection tube (3ml)**	1	1 ml whole blood aliquot per 2.0 ml cryovial (green cap)	3	Frozen
Whole blood for RNA extraction	**PAXgene™ Blood Collection Tube (2.5 ml)**	**1**	**N/A**	**1**	Frozen
Whole blood for PBMC *(*optional)*	**Sodium Heparin (Green-Top) Blood Collection tube (10 ml)**	**2**	**N/A**	**N/A**	N/A – Kept locally at site

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
CSF Collection ***(*not collected at 48-Month/Annual visit)***	**Sterile Container**	Conical tubes, 15 cryovial tubes (13 orange cap, 1 blue cap, 1 yellow cap)	1.5 ml CSF aliquots per 2.0 ml cryovial (orange cap); residual volume placed in 2.0 ml cryovial with blue cap; 1-2 ml for local lab placed in 2.0 ml cryovial with yellow cap.	Up to 14	Frozen

5.2.2 Biofluid Collection for CN Participants: Baseline, 12-Months, 24-Months, 36-Months, and 48/Annual visits.

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
Whole blood for isolation of serum	**Plain Red-Top Serum Blood Collection Tube (10 ml)**	**1**	**N/A**	**N/A**	**N/A**
	SERUM: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	4	1.5 ml serum aliquot per 2.0 ml cryovial (red cap)	Up to 4	Frozen
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	**3**	**N/A**	**N/A**	**N/A**
	PLASMA: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	10	1.5 ml plasma aliquot per 2.0 ml cryovial (lavender cap)	Up to 10	Frozen
	BUFFY COAT: 2.0 ml cryovial	3	1 ml buffy coat aliquot per 2.0 ml cryovial (clear cap)	3	Frozen
Whole blood for long read sequencing ***(*collected only once)***	**EDTA (Lavender-Top) Blood Collection tube (3ml)**	1	1 ml whole blood aliquot per 2.0 ml cryovial (green cap)	3	Frozen
Whole blood for RNA extraction	**PAXgene™ Blood Collection Tube (2.5 ml)**	**1**	**N/A**	**1**	Frozen

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
Whole blood for PBMC *optional	**Sodium Heparin (Green-Top) Blood Collection tube (10 ml)**	**2**	**N/A**	**N/A**	N/A – Kept locally at site
CSF Collection *(*collected at BL and M24, may be collected at M12 if CSF was not collected at BL)*	Sterile Containers (cryovial with yellow cap)	Conical tubes, 15 cryovial tubes (13 orange cap, 1 blue cap, 1 yellow cap)	1.5 ml CSF aliquots per 2.0 ml cryovial (orange cap); residual volume placed in 2.0 ml cryovial with blue cap; 1-2 ml for local lab placed in 2.0 ml cryovial with yellow cap.	Up to 14	Frozen

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-C**).** Submit a copy to NCRAD with a reason provided for the omission and track it as a protocol deviation.

6.0 Specimen Collection Kits, Shipping Kits, and Supplies

NCRAD will provide the kits and individual supplies listed in section 6.1. Itemized kit contents can be found on the iLEADS Kit Request Module.

6.1 Kits and Supplies Provided by NCRAD

Do not replace or supplement any of the 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.*

6.1.1 iLEADS CI Baseline Blood Kit

This kit comes with the supplies necessary for the collection of:

- Whole blood for isolation of serum
- Whole blood for isolation of plasma & buffy coat
- Whole blood for CLIA genetic testing
- Whole blood for isolation of RNA
- Whole blood for isolation of PBMC

6.1.2 iLEADS CI M12 – M72 and CN BL – M72 Blood Kit

This kit comes with the supplies necessary for the collection of:

- Whole blood for isolation of serum
- Whole blood for isolation of plasma & buffy coat
- Whole blood for isolation of RNA
- Whole blood for isolation of PBMC

6.1.3 *iLEADS Long Read Sequencing Blood Kit*

This kit comes with the supplies necessary for the collection of:

- Whole blood for long read sequencing

6.1.4 *iLEADS Supplemental Blood Kit*

This kit comes with extra blood collection supplies to have on hand.

6.1.5 *iLEADS LP Kit*

This kit comes with a 24G LP tray for CSF collection

6.1.6 *iLEADS CSF Kit (CI & CN BL –M36)*

This kit comes with the supplies necessary for the collection of CSF.

- Note: for every CSF draw a LP Kit and a CSF Kit must be ordered.

6.1.7 *iLEADS Supplemental CSF Kit*

This kit comes with extra CSF collection supplies to have on hand.

6.1.8 *iLEADS Supplies*

The kit request module has a list of supplies that can be ordered individually, if ever needed.

6.2 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 follow the applicable link below 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.

- iLEADS Sites: https://redcap.uits.iu.edu/surveys/?s=DRE4RPARK3R7D8KL

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.

7.0 Blood Collection and Processing Procedures

***Important Note**: In order to ensure the highest quality samples are collected, processed, and stored, it is essential to follow the specific collection, processing, and shipment procedures detailed in the following pages. Collection of biomarkers and CSF should be collected after a minimum 6-hour fast, preferably in the morning. Refer to the main study protocol for more information regarding fasting prior to CSF collection. Please read the following instructions first

before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood. Please note that the centrifuge may take 30 minutes to cool, so please plan accordingly. Draw blood in the following order:

1. Plain Red Top Serum Blood Collection Tube (10 ml) for Serum
2. EDTA (Lavender-Top) Blood Collection Tube (10 ml) for DNA and Plasma x 3
3. EDTA (Lavender-Top) Blood Collection Tube (6 ml) for CLIA lab testing
4. EDTA (Lavender-Top) Blood Collection Tube (3 ml) for long read sequencing.
5. PAXgene™ Blood Collection Tube (2.5 ml) for RNA
6. *Optional:* Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)* x 2

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

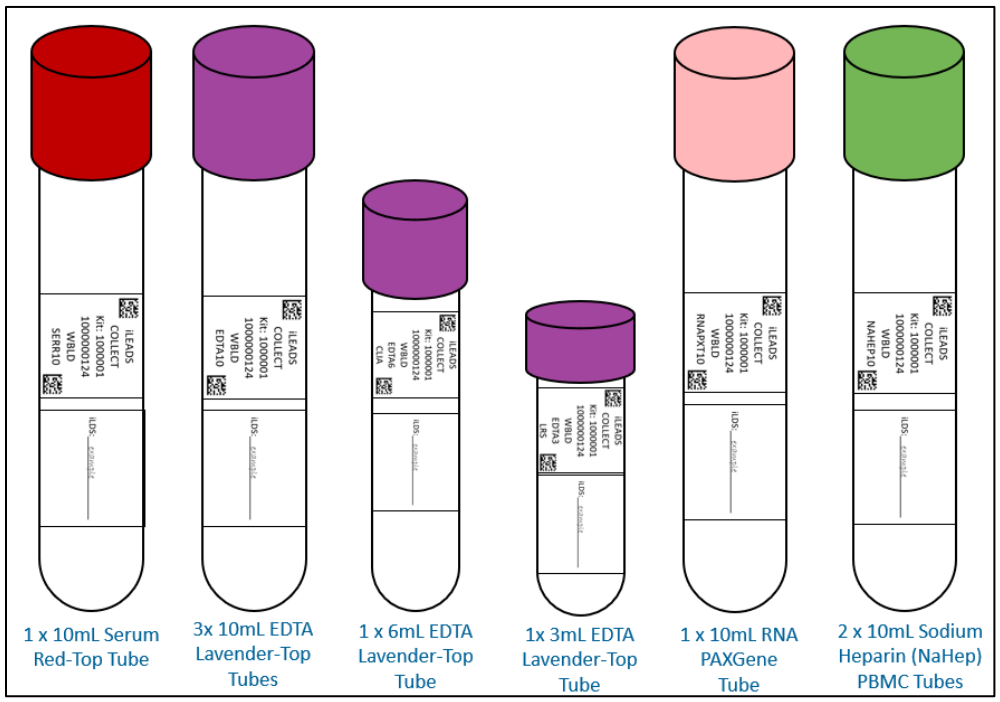
7.1 Kit Supply to Study Sites

Label Type Summary:

1. Kit Number Label
2. Collection Tube Label
3. Aliquot Tube Label
4. iLEADS ID Label

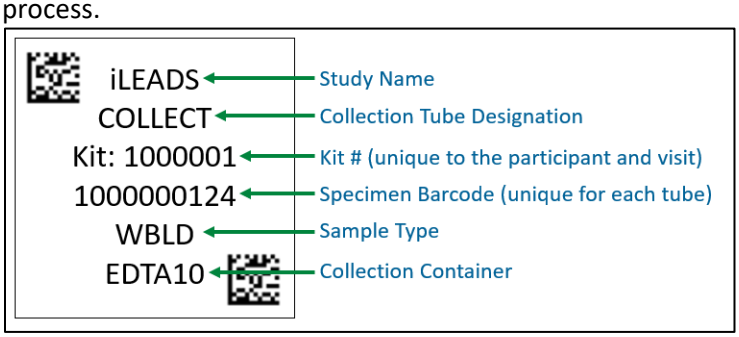
Kit Number 1000001	The **Kit Number Labels** do not indicate a specimen type but are affixed on the Biological Sample and Shipment Notification Forms and on specific packing materials.
iLEADS COLLECT Kit: 1000001 1000000124 WBLD SERR10	The **Collection Tube Labels** for blood and CSF are placed on all collection tubes.
iLEADS ALIQUOT Kit: 1000001 1000000124 SER SERR10	The **Aliquot Tube Labels** for blood, blood derivatives and CSF are placed on all aliquot tubes.
iLDS:_____	The **iLDS ID Labels** are placed on all collection tubes, both blood and CSF.

***Important Note**: **Each collection tube will contain two labels**: the Collection and Aliquot Tube Label and the iLDS 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 iLDS ID label.

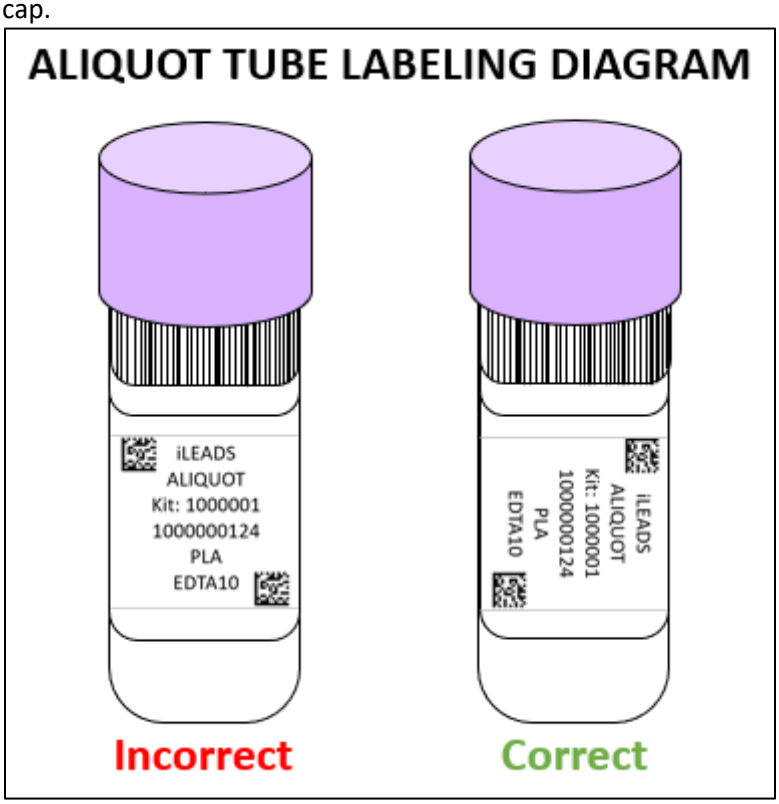


In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place blood collection and aliquot labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing, or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Place cryovials in numerical order based on the specimen number, located at the top of the label. This ensures that no aliquot is misplaced or lost during the shipment process.



- Using a fine point permanent marker, fill-in and place the iLDS ID Labels on the collection tubes only (RNA, Serum, NaHep, EDTA(s)) **BEFORE** sample collection, processing, or freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place iLDS ID labels on any cryovials.
- Using a fine point permanent marker, fill-in and place the iLDS ID Labels on the collection tubes only **BEFORE** sample collection, processing, or freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place iLDS ID labels on any cryovials.
- The Collection and Aliquot Tube Labels contain two 2D barcodes on the upper left and lower right-hand sides of the label. Place the left-hand barcode toward the tube cap.



- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see labeling diagram below).
- Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label.
- If there are any unused cryovials, please do not send the empty cryovials to NCRAD. These unused cryovials (ensure labels are removed) can be saved as part of a supplemental supply at your site or the cryovials can be disposed of per your site's requirements.

7.2 Filling Aliquot Tubes (Serum, Plasma, Buffy Coat, Whole Blood, and CSF)

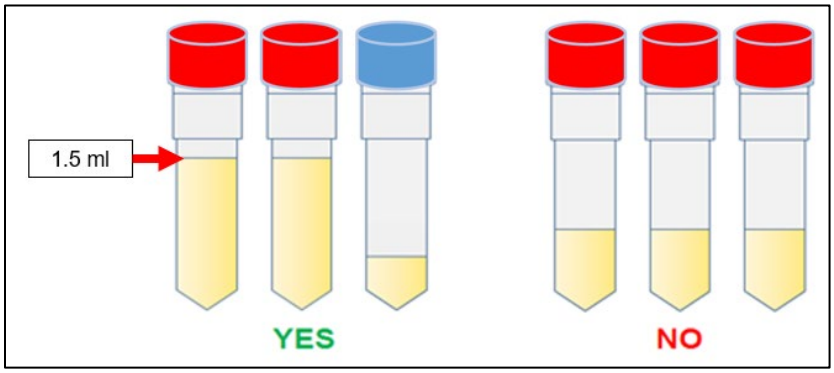
In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each cryovial should be filled to the assigned volume with the respective biological material after processing is completed (refer to detailed processing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

- Serum, plasma, and CSF samples should be processed into 1.5ml aliquots.
- Buffy coat and whole blood (for long read sequencing) should be processed into 1.0ml aliquots.

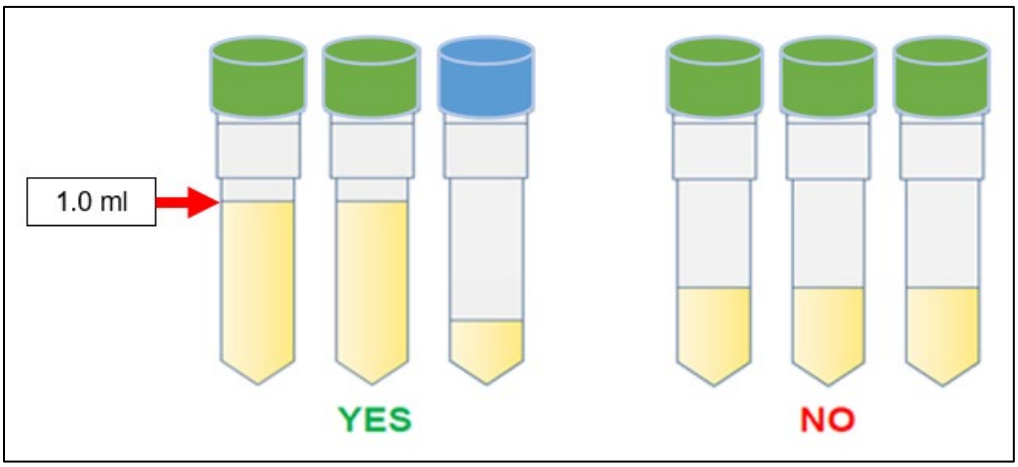
Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample.

Residuals: If applicable, 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. Serum, plasma, whole blood (for long read sequencing), and CSF samples may require a residual. Residuals for serum, plasma, and CSF samples should be created if there is less than 1.5ml of sample remaining after filling all other aliquot tubes. A residual for whole blood (for long read sequencing) should be created if there is less than 1.0ml of sample remaining after filling all other aliquot tubes.

For example, if 3.6 ml of ***serum*** is obtained, you should fill 2 red capped cryovial tubes each with **1.5 ml**, and one additional blue capped cryovial tube with the remaining 0.6 ml.



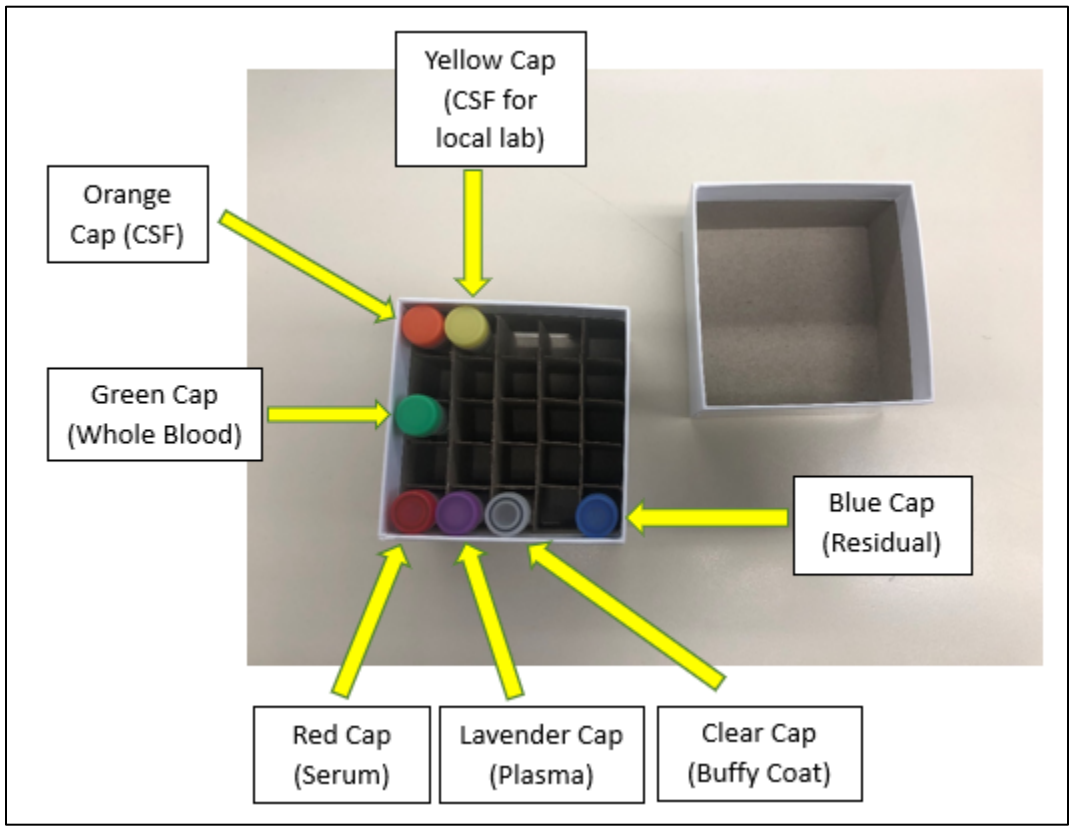
Another example, if 2.7 ml of ***whole blood (for long read sequencing)*** is obtained, you should fill 2 green capped cryovial tubes each with **1.0ml**, and one additional blue capped cryovial tube with the remaining 0.7 ml.



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 1.5 ml for serum, plasma, or CSF samples and anything under 1 ml for whole blood aliquots). 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 cryovial tubes. The chart below summarizes the association between cap color and type of cryovial.

Cap Color	Sample Type
Red Cap	Serum
Lavender Cap	Plasma
Clear Cap	Buffy Coat
Green Cap	Whole blood
Blue Cap	Residual (plasma, serum, whole blood or CSF)
Orange Cap	CSF
Yellow Cap	CSF for local lab


Yellow Cap (CSF for local lab)
Orange Cap (CSF)
Green Cap (Whole Blood)
Blue Cap (Residual)
Red Cap (Serum)
Lavender Cap (Plasma)
Clear Cap (Buffy Coat)

7.3 Plain Red-Top Serum Blood Collection Tube (10 ml) for Serum

Whole Blood Collection for Isolation of Serum: Plain Red-Top Serum Blood Collection Tube (10 ml) (for processing of serum aliquots)

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

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

2. Place completed iLDS ID Label and Collection **"WBLD SERR10"** Tube Label on the Plain Red-Top Serum Blood Collection Tube. Place pre-printed Aliquot "**SER SERR10**" Tube Labels on the (3) 2.0 ml cryovial tubes with red caps and (1) 2.0 ml cryovial with blue cap (if necessary, for residual).

3. Using a blood collection set and a holder, collect blood into **Plain Red-Top Serum Blood Collection Tubes (10 ml)** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:
   a) Place 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 last collection tube.
   d) Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.

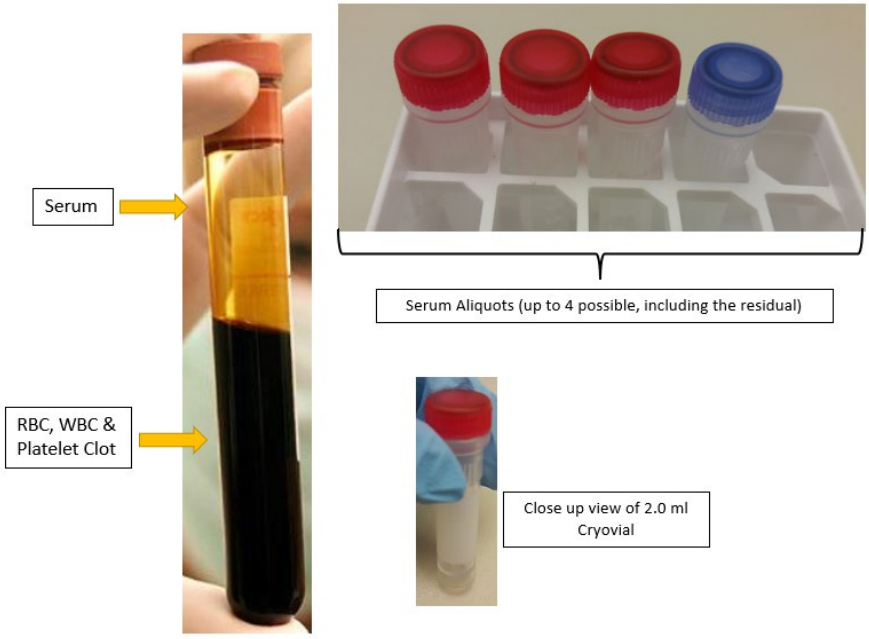
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into each tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
   a. If complications arise during the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

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

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

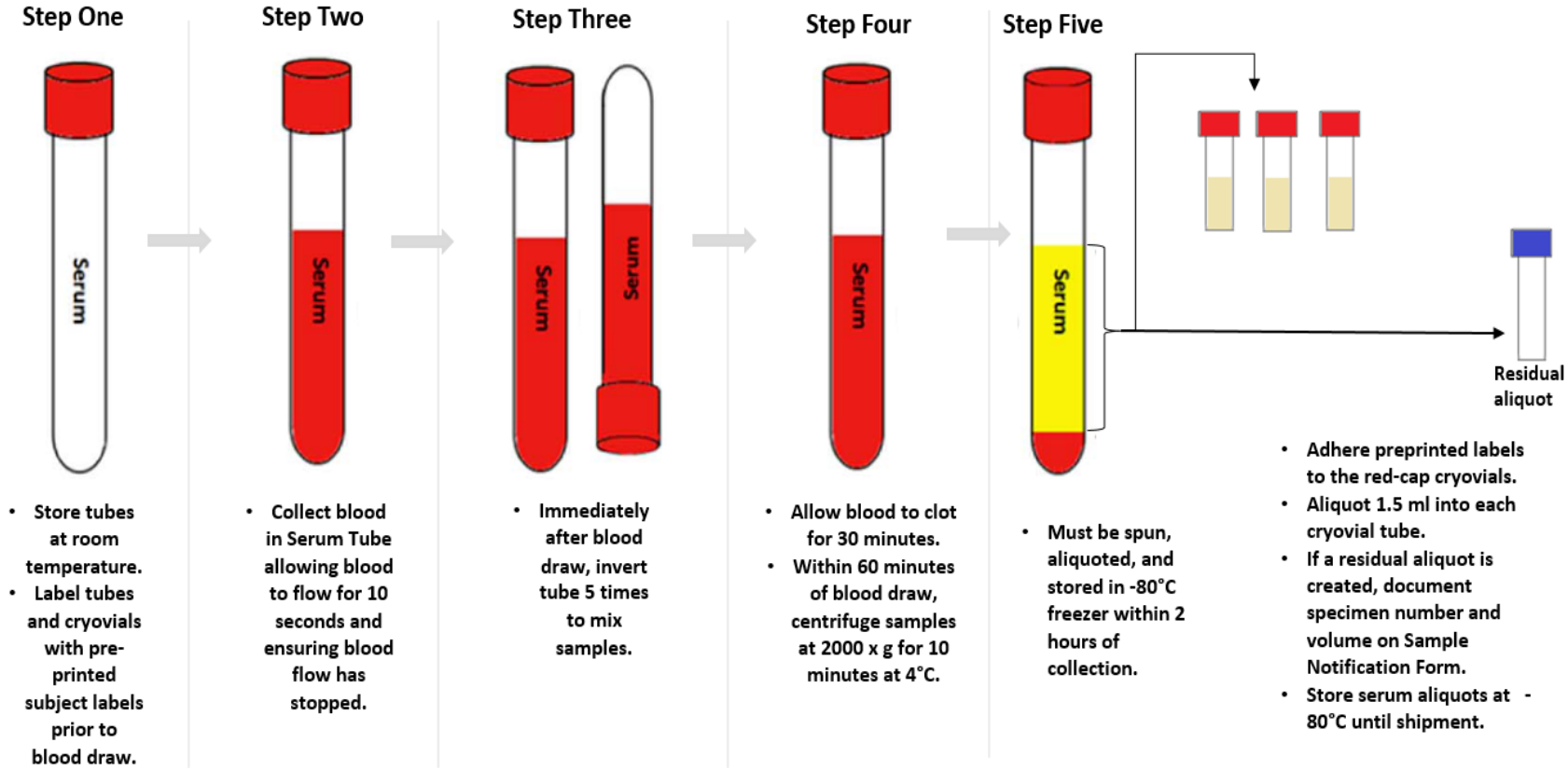
7. After 30 minutes of clotting, centrifuge the collection tube for 10 minutes at 2000 x g at 4°C. **It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum separation (see worksheet in Appendix A to calculate RPM)**
    a. Equivalent rpm for spin at 2000 x g
    b.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

8. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the wall. Using a disposable pipette, transfer serum into the pre-labeled cryovials with the red caps. Aliquot 1.5 ml per cryovial (total vials= up to 3 with 1.5 ml each and 1 residual with <1.5 ml). The Serum tube should yield, on average, 4- 5 ml of serum for a total of (3) 2.0 ml aliquot cryovial tubes per participant with 1.5 ml per cryovial tube. Be sure to only place **serum** in cryovials labeled with the "SERUM" label and red caps. If there is extra serum left, use 1 extra cryovial provided 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.**



9. Place the labeled cryovials in the 81 cell cryobox and place on dry ice pellets. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice pellets. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

Serum Preparation (10ml Red Top Tube)



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

7.4 EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Plasma and Buffy Coat

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

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

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

2. Place completed iLDS ID Label and pre-printed Collection "**WBLD EDTA10**" Aliquot Tube Labels on the 3 x 10mL lavender top EDTA tubes. Place pre-printed Aliquot "**PLA EDTA10**" Aliquot Tube Labels on the (9) 2.0 ml cryovial tubes with lavender caps and (1) 2.0 ml cryovial tube with blue cap (if necessary, for residual). Place pre-printed Aliquot "**BUF EDTA10**" Aliquot Tube Labels on the (3) 2.0 ml cryovial tubes with clear lids.

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

4. Using a blood collection set and a holder, collect blood into the **EDTA (Lavender-Top) Blood Collection Tube (10 ml)** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:
   a) Place 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 last collection tube.
   d) Make sure tube additives do not touch stopper or end of the needle during venipuncture.

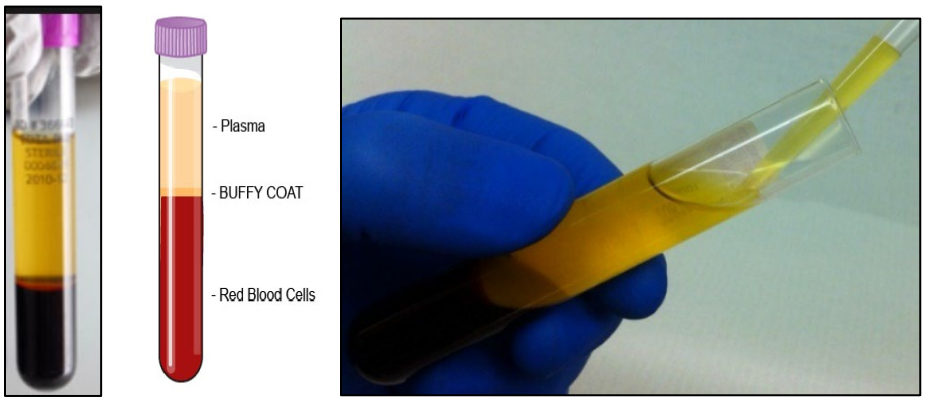
5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
   a. If complications arise during the blood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

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

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

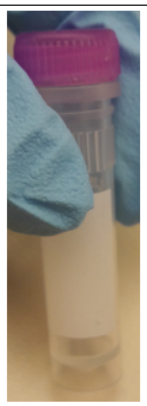
8. Preferably within 30 minutes of blood collection, centrifuge balanced tubes for 10 minutes at 2000 x g 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM.)**
   a. Equivalent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

9. 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 three EDTA tubes into the 50 ml conical tube and gently invert 3 times. Aliquot 1.5 ml per cryovial (total vials = up to 9 with 1.5 ml each and 1 residual with <1.5ml). The EDTA tube should yield, on average, 4-5 ml of plasma. Be sure to only place **plasma** in cryovials with lavender caps and labeled with "PLASMA" labels**.** Take caution not to disturb the red blood cells at the bottom of the tube. If there is extra plasma left, use 1 extra cryovial provided for another <1.5 ml aliquot of plasma. **If a residual aliquot (<1.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**



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

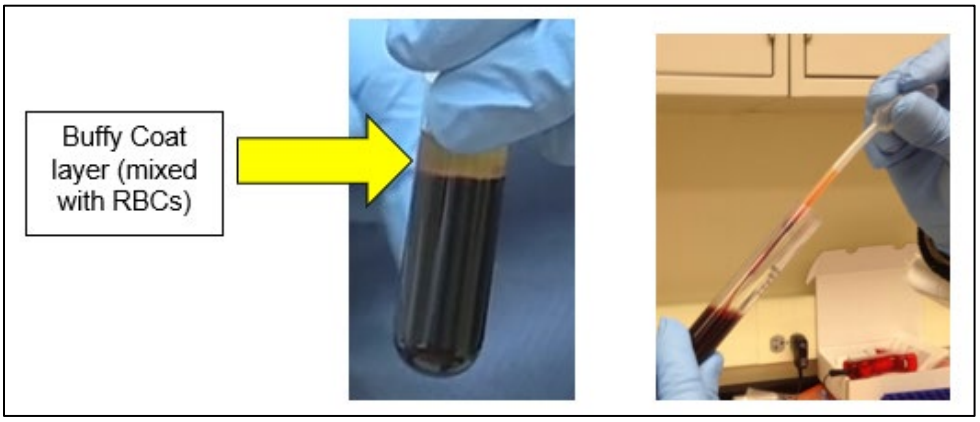
Plasma Aliquots (10 possible)



Close up view of 2.0 ml Siliconized Cryovial

10. Place the labeled cryovials in the 81 cryovial box and place on dry ice pellets. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice pellets. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample Shipment and Notification Form.

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

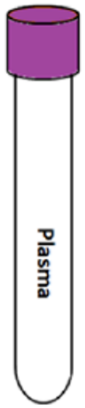


12. Dispose of tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.

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

Plasma and Buffy Coat Preparation (10ml Lavender-Top Tube x 3)

Step One

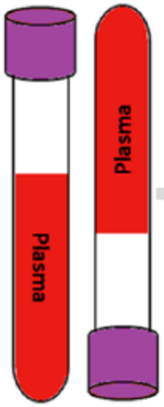


- Store tubes at room temperature.
- Label tubes with preprinted labels prior to blood draw.

Step Two

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

Step Three



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

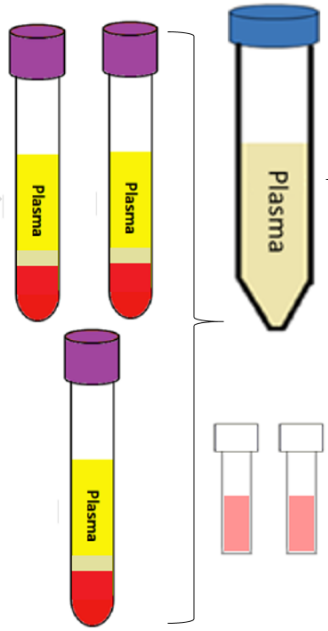
Step Four

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

Step Five

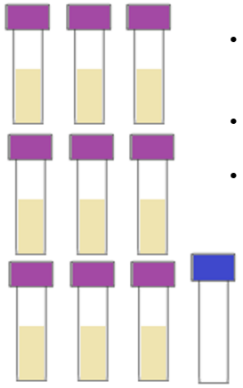
- Preferably within 30 minutes, centrifuge samples at 2000 x g at 4°C for 10 minutes.
- Samples need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.

Step Six



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

Step Seven



- Adhere preprinted labels to the lavender cap cryovials.
- Aliquot 1.5 ml into each cryovial tube.
- If a residual aliquot is created, document specimen number and volume on Sample Notification Form. Store plasma aliquots at -80°C until shipment.

Residual aliquot

Step Eight

- Adhere preprinted labels to the clear cap cryovials.
- Using a clean pipette tip, collect the buffy coats (may have residual plasma and some RBCs included).
- Transfer the buffy coats into the cryovial tubes.
- Store buffy coat aliquots at -80°C until shipment.

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

7.5 EDTA (Lavender-Top) Blood Collection Tube (6ml) for CLIA Lab Testing

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

1. CRITICAL STEP: **Store empty Whole Blood EDTA tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.**

2. Place completed iLDS ID Label and pre-printed "**WBLD EDAT6**" Collection Tube Label on the 6ml lavender-top EDTA tube.

3. Using a blood collection set and a holder, collect whole blood into the 6 ml lavender top whole blood tube using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

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

4. Invert the tube gently 3 times.

5. Transfer the tube immediately to a **-80°C Freezer.** The sample should be frozen and stored **UPRIGHT** in a WIRE or PLASTIC type test tube rack (DO NOT use a solid Styrofoam test tube holder).

Whole Blood Preparation (6 mL Lavender-Top Tube)

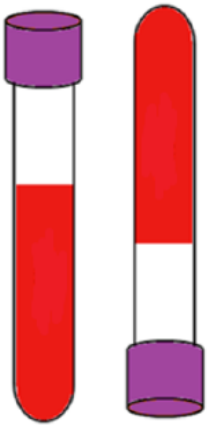
Step One

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

Step Two

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

Step Three



- Immediately after blood draw, invert tube 3 times to mix sample.

Step Four

- Immediately after inversion, freeze the sample in an -80°C freezer until ready to ship.

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

7.6 EDTA (Lavender-Top) Blood Collection Tube (3ml) for Whole Blood x 1

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

1. CRITICAL STEP: **Store empty Whole Blood EDTA tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.**

2. Place completed iLDS ID Label and pre-printed "**WBLD EDTA3**" Collection Tube Label on the 3ml lavender top EDTA tube. Place the (3) "**WBLD EDTA3**" Aliquot Tube Labels on the (3) 2.0 ml cryovial tubes with green caps (or (2) 2.0ml cryovial tubes with green caps and (1) 2.0ml cryovial tube with blue cap if a residual is created instead of a full third aliquot).

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

4. Using a blood collection set and a holder, collect whole blood into the 3 ml lavender top whole blood tube using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:

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

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

6. Using a disposable pipette, transfer whole blood into the pre-labeled cryovials with green caps. Aliquot 1 ml of whole blood per cryovial (total vials = up to 3 with 1 ml each). Be sure to only place whole blood in cryovials with green caps and labeled with "**WBLD**" labels.
   a. If the third aliquot contains less than 1ml of sample, place the blue cap on the cryovial.

7. Place the labeled cryovials in the 81 cryovial box and place on dry ice pellets. Transfer to -80°C Freezer when possible. Store all samples at -80°C until shipped to NCRAD on dry ice pellets. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample Shipment and Notification Form.

Whole Blood Collection (1 x 3ml EDTA Purple Top Tube)

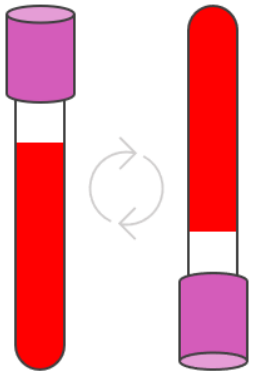
Step 1

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

Step 2

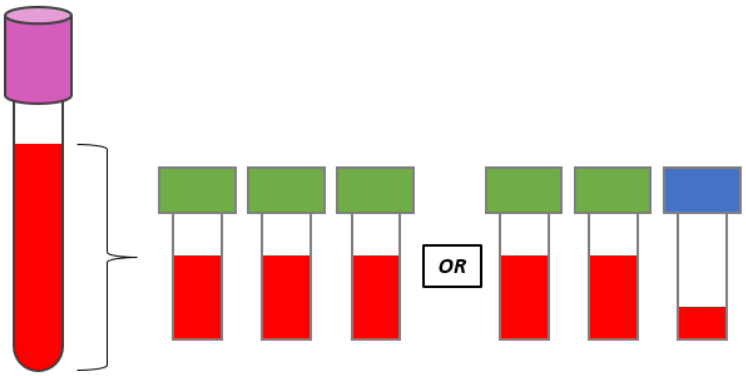
- Collect blood in EDTA Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step 3



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

Step 4



- Adhere preprinted labels to the green cap cryovials.
- Aliquot 1 ml into each cryovial tube.
- If a residual aliquot is created, document specimen number and volume on Sample Notification Form.
- Store whole blood aliquots at -80°C until shipment.

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

7.7 2.5 ml PAXgene™ Tube for RNA

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

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

1. Place filled-out iLDS ID Label and Collection "**WBLD RNAPXT10**" Tube Label on the PAXgene™ tube prior to blood draw; no processing is required for this tube**. <u>The single tube is to be shipped to NCRAD frozen, without processing at the collection site.</u>**

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

3. Using a blood collection set and a holder, collect blood into the **PAXgene™ RNA Tube** using your institution's recommended procedure for standard venipuncture technique.

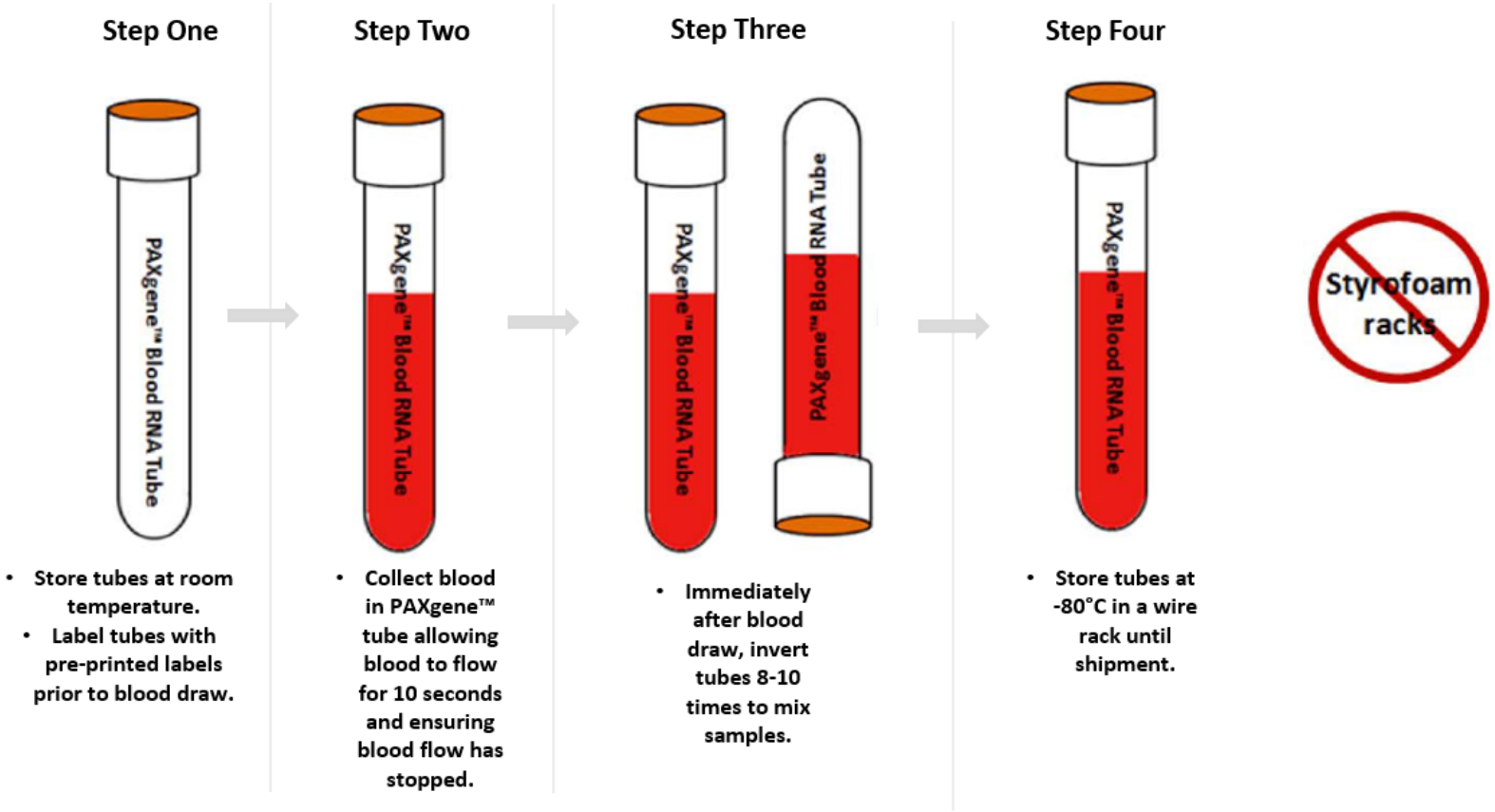
   The following techniques shall be used to prevent possible backflow:
   a) Place participant's arm in a downward position.
   b) Hold tube in a vertical position, below the participant's arm during blood collection.
   c) Release tourniquet as soon as blood starts to flow into last collection tube
   d) Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into 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 **<u>WIRE</u>** 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 using dry ice pellets to NCRAD. Complete remainder of the Biological Sample and Shipment Notification Form (Appendix B).

RNA Preparation (2.5ml PAXgene™ Tube)



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

7.8 Sodium Heparin (Green-Top) Blood Collection Tube (10 ml) for Collection of Peripheral Blood Mononuclear Cells (PBMC) x 2

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

***Important Note:** PBMC collection is optional for iLEADS sites. If collected, PBMC samples will be stored locally, at the iLEADS sites. PBMC samples will not be sent to NCRAD.

1. **CRITICAL STEP: Store empty Sodium Heparin tube at room temperature, 64°F - 77°F (18°C to 25°C) before use.**

2. Place completed iLDS ID Label and pre-printed "**WBLD NAHEP10**" Collection Tube Label on the green-top NaHep tubes. **Please ensure that a full vertical (from cap to bottom) strip of the tube remains unobstructed.**

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

   The following techniques shall be used to prevent possible backflow:

   a) Place participant's arm in a downward position.

   b) Hold tube in a vertical position, below the participant's arm during blood collection.

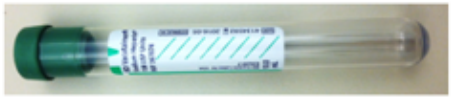
   c) Release tourniquet as soon as blood starts to flow into last collection tube.

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

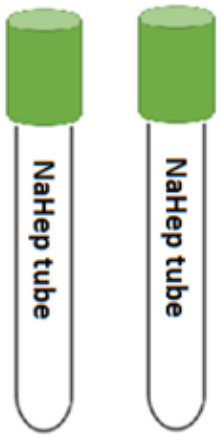
4. Immediately after blood collection, gently invert the tubes 8-10 times to mix sample.

5. Store and process samples locally on site. Do not send the samples to NCRAD.

PBMC Preparation (10ml Sodium Heparin Tube x 2)

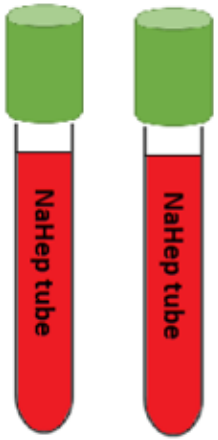


Step One



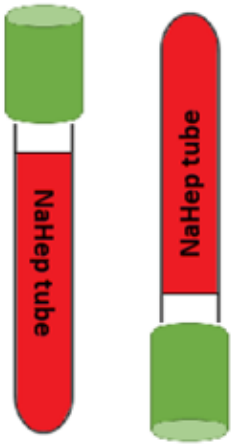
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



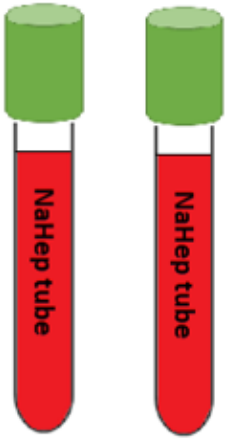
- Collect blood in Sodium Heparin Tubes allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four



- Process samples locally at site and do not send to NCRAD

Please be sure to compare the labels on each tube and cryovials to the Biological Sample Form included with each kit

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

8.0 Cerebrospinal Fluid Collection and Processing

8.1 Previously Collected CSF

International sites may have stored CSF that was collected previously for other EOAD studies or routine assessments in clinic. To avoid repeat lumbar puncture procedures and reduce participant burden, international sites may send these pre-existing CSF samples to LEADS if collected within the 12 months prior to a participant's consent date and approval is received by the Genetics and Biorepository Core. Applicable language must be included in the site's consent form. If approval is obtained for LEADS, these samples can be shipped to NCRAD.

When this scenario arises, the site coordinator should contact NCRAD (agericks@iu.edu) to prepare the previously collected CSF for shipment. The site will work with NCRAD to ensure that samples meet NCRAD requirements. Do not ship to NCRAD until receiving direct approval from NCRAD staff.

8.2 General Information for New CSF Collection

***Important Note**: CSF samples should be collected in the morning before breakfast and after an overnight fast. There should be a minimum **6-hour fast** before collection of biomarker fluids and CSF. Only water is permitted until blood draws and the lumbar puncture are completed.

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

- Begin by confirming participant consented to lumbar puncture (LP) before scheduling the procedure and again prior to performing procedure.
- If LP and PET scan are done on the same day, LP should be completed prior to the PET scan; otherwise, there should be at least 12 hours between LP and PET scan.
- Do NOT use any extension tubing due to the tendency of manufactured plastic tubing to bind beta amyloid peptides and other important AD biomarkers.
- If LP was attempted but unsuccessful in obtaining CSF, a second attempt under fluoroscopy (if deemed appropriate by site clinician) is allowed.
- LP under fluoroscopy is permitted, if needed. Site personnel should advise the participant that use of fluoroscopy (x-rays) involves exposure to radiation.
- Participants taking an anti-platelet agent (e.g. aspirin) may, at the discretion of the site clinician, be discontinued from that agent for a period of time prior to lumbar puncture and/or continue off agent for a period of time post LP. Participants who are taking anticoagulants (e.g. warfarin (Coumadin) and/or dabigatran (Pradaxa)) may not undergo an LP and are not suitable to participate in this study.
- Each study participant or a person designated to speak for them will be contacted by phone within 48 hours after the LP to confirm participant well-being and to query about any adverse events.
- Identify a physician (e.g., anesthesiologist) able to perform a blood patch for any participant who experiences a post lumbar puncture headache. Find out ahead of time

who to call to schedule and perform a blood patch at your center, should the need arise. Ensure billing procedures are in place ahead of time.

- Ensure you have at least two "Lumbar Puncture Tray Kits" and sufficient "CSF Supplemental Supply Kit" provisions on hand prior to scheduling an LP visit. Also ensure adequate site-provided supplies (see above), including pelleted dry ice. Check expiration dates on all supplies, especially lidocaine.

8.3 Scheduling the LP

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

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

8.4 Performing the LP

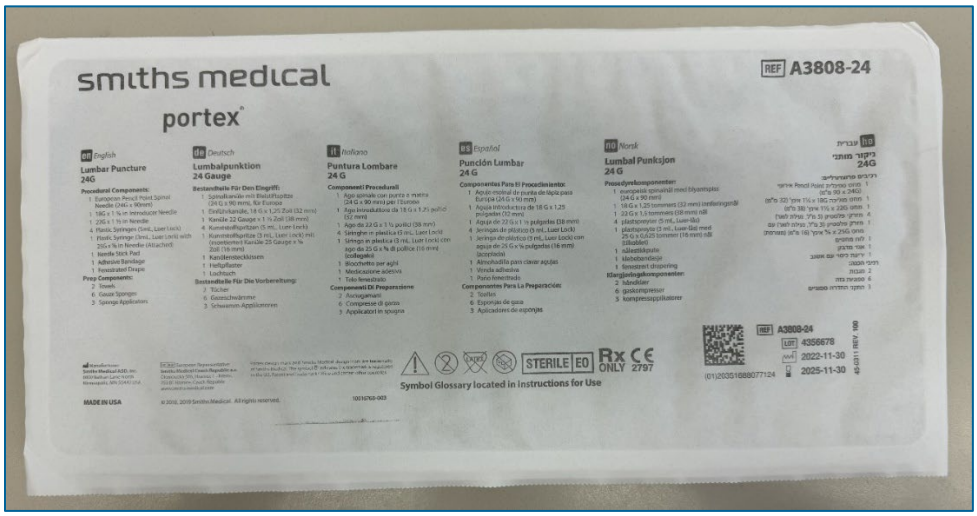
The recommended position is sitting. The same position should be used at follow-up LPs. It is critical to try to optimize positioning, and usually requires an assistant. Other positions and needles are allowed (e.g., when using fluoroscopy) but this should be recorded on the CSF Sample and Shipment Notification Form.

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

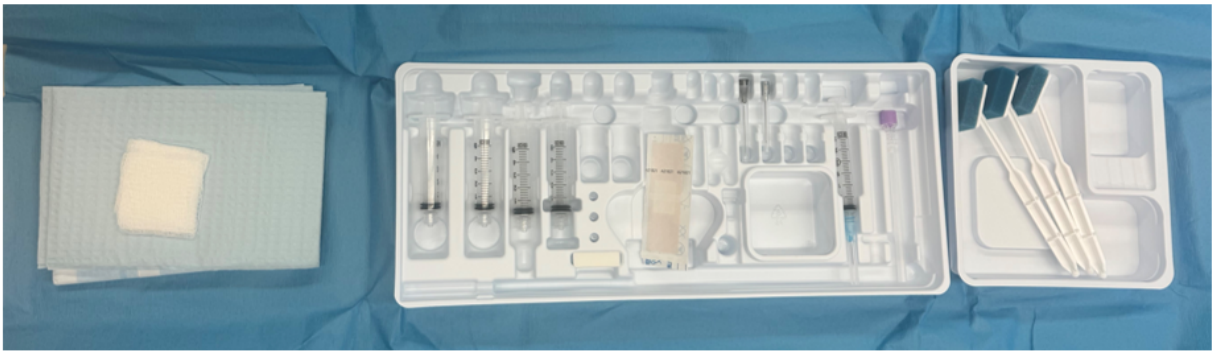
Feel the outside of the lumbar puncture kit (still wrapped up) to determine which end contains the spongy swabs. Turn this end toward the person performing the lumbar puncture and begin un-wrapping the kit.

8.5 LP Tray Kit Images

Exterior of a 24G LP Tray provided by NCRAD:



Interior of a 24G LP Tray provided by NCRAD:



Interior of a 24G LP Tray provided by NCRAD

Procedural Components:	Prep Components:
1 - European Pencil Point Spinal Needle (24G x 90mm)	2 - Towels
1 - 18G x 1 ¼ in Introducer Needle	6 - Gauze Sponges
1 - 22G x 1 ½ in Needle	3 - Sponge Applicators
4 - Plastic Syringes (5mL)	
1 - Plastic Syringe (3mL) with 25G x 5/8 in Needle (Attached)	
1 - Needle Stick Pad	
1 - Adhesive Bandage	
1 - Fenestrated Drape	

TOUCH ONLY THE OUTSIDE OF THE PAPER WRAPPER

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

- If you touch any part of the paper wrapper, or if any non-sterile object outside of the wrapper touches any part of the inside of the wrapper, throw the kit away and start over.
- If you are in any doubt as to whether the inside of the wrapper has been touched, throw the kit away and start over.

Cleaning the Lumbar Puncture Site
The lumbar puncture site is cleaned with Povidone-Iodine Topical Solution according to best standard medical practices.

Once the kit is successfully unwrapped, open the bottle of Povidone-Iodine Topical Solution somewhere away from the kit. Use an alcohol swab to remove any loose chunks of dried material off the bottle top. You don't want anything to fall onto the open and sterile lumbar puncture kit. Pour enough Povidone-Iodine Topical Solution into the prep well to cover the bottom, about ¼ inch deep.

Maintaining the Sterile Field
An important aspect of assisting with a successful lumbar puncture is keeping the field sterile. If there are multiple staff members in the room, please be sure they do not accidentally contaminate the sterile field. Once the person performing the lumbar puncture has donned sterile gloves, additional help may be needed to obtain or un-wrap any new tubes, needles, or supplies.

Unwrapping the Sterile 15- and 50-ml Conical Tubes
Note that the 15-ml and 50-ml tubes into which CSF is collected and transferred come individually wrapped and are sterile inside and out. These wrappers should be peeled open by an assistant (not touching the tube) and the tube carefully dropped onto the LP tray or elsewhere in the sterile field in a manner that avoids contamination. Any additional needles or other individually wrapped sterile items can be handled the same way.

- Do not drop any packaging onto the tray or sterile field.
- Do not let the item touch the outside of the packaging on its way to the tray.

Lidocaine, Syringe with Needle, Gauze Pads
Anesthesia is usually achieved within 2 minutes after injecting the lidocaine. Occasionally, the person performing the lumbar puncture will need to use more lidocaine to numb up a particular spot, or they may need to move to another spot entirely.

Next, hold the lidocaine bottle upside down and at a slight angle toward the person performing the lumbar puncture so that they can plunge the needle into the bottle and extract some lidocaine without touching you or the bottle. Use two hands to stabilize the bottle. If the person performing the LP requires additional sterile gauze, open the gauze pad the same way as the syringe and needle, by holding open the package so the

person performing the lumbar puncture can grab the gauze without touching you or the package.

General CSF Collection Methods

LPs for CSF collection should be performed using a small caliber atraumatic needle. CSF should be obtained via gravity flow using the 24-gauge Sprotte needle, although aspiration through this or smaller needles is allowable. Prior approval from the Clinical Core is required before the aspiration method can be utilized. Sites must designate the method of CSF collection for data tracking purpose. It is recommended that CSF be obtained from participants in a sitting position. Alternate needles, positions, or methods (e.g., use of fluoroscopy) should be noted on the CSF Sample and Shipment Notification Form.

Collection of CSF by Gravity
After the spinal needle is placed in the intrathecal space and the stylet is withdrawn, CSF should flow freely. **Discard first 1-2mls of CSF if blood tinged. If not blood tinged, collect first 1-2 mLs of CSF into a 15ml conical tube and pipette into the yellow cap cryovial for local lab. Collect 15-20ml CSF total into the remaining (2) 15ml conical tubes.**

Reminder: If the CSF is blood-tinged, the first 1-2 ml of CSF should be discarded (or more if needed) to clear the blood before collecting the 10-20 ml for CSF analysis. 10 ml is the required MINIMUM for CSF biomarker analysis. If 10 ml is not obtained and provided to the NCRAD, document the reason for under-collection on the comments section of the CSF Sample and Shipment Notification Form.

Up to 20ml of CSF can be collected for the iLEADS protocol. Any additional CSF collected will require a separate informed consent document that is connected to a specific protocol. We recommend that the additional non-iLEADS CSF collected does not exceed 10ml for a total of 30ml.

Washcloths, Band-Aids, and Clean Up
After the person performing the lumbar puncture collects the last of the CSF, remove the needle and introducer and wash the Povidone-Iodine Topical Solution off the participant. A warm, wet washcloth can be used. A Band- Aid should be applied to the puncture site. Next, discard the LP kit following local guidelines, and dispose of sharp components in an appropriate sharps container.

Step by Step Summary of CSF Collection Procedure
1. Ensure all samples collected are appropriately labeled.

2. Print CSF Sample and Shipment Notification Form.

3. Confirm all supplies, including pelleted dry ice and wet ice, are available.

4. Label the (13) orange cap cryovials and (1) blue cap cryovial with provided iLEADS CSF labels. Do **NOT** open and label the 15-ml and 50-ml tubes that will be kept sterile to collect the CSF.

5. Pre-cool the centrifuge and pre-cool all (14) labeled tubes on wet ice. Do **NOT** pre-cool the 15-ml and 50-ml tubes that will be kept sterile to collect the CSF.

6. Measure vitals (participant lying down).

7. Record the time of LP and associated information on the CSF Sample and Shipment Notification Form.
8. Collect 10-20 ml CSF at the L3/L4 position (or adjacent position) using a 24 gauge Sprotte spinal needle via gravity flow with participant in upright position (or document alternate method on CSF Sample and Shipment Notification Form) following these steps:
    a. Collect initial 1-2 ml (if bloody, collect CSF until cleared of blood) using the 15ml conical tube. If not bloody, transfer first 1-2ml into yellow cap cryovial for local lab.
    b. Collect an additional 10-20 ml CSF into the **UNLABELED-STERILE** 15-ml polypropylene tubes from the "CSF Supply Kit". 10 ml is the required **MINIMUM.**
    c. If using aspiration, use **ONLY** the polypropylene syringes included in the "Lumbar Puncture Collection Kit" and transfer **DIRECTLY** into the **UNLABELED-STERILE** 15-ml polypropylene tube from the "CSF Supply Kit". There are four 6 ml Luer lock polypropylene syringes in the "Lumbar Puncture Collection Kit." Note this on the CSF Sample and Shipment Notification Form.

9. As one person takes the immediate post procedure vital signs, a second person should process the CSF as follows:
    a. Place samples upright on wet ice and ensure samples are kept on wet ice for the entire time prior to processing. Preferably within 15 minutes of collection, centrifuge briefly at low speed (2000 x g, 10 min, 4°C) to pellet any cellular debris.
    b. Using a clean transfer pipette, transfer CSF from both 15ml conical tubes into a 50ml conical tube, leaving the debris at the bottom of each 15ml centrifuged tube. Gently invert the 50ml conical tube 3-4 times to mix the sample.
    c. Aliquot 1.5ml into the orange-cap cryovials. If a residual aliquot is created, aliquot into blue cap cryovial. Document specimen number and volume on CSF Sample Notification Form.
    d. Within 2 hours of CSF collection, samples need to be spun, aliquoted, and in the freezer. Store CSF aliquots at -80^{o}C until shipment. Record time of freezing on CSF Sample and Shipment Notification Form.

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

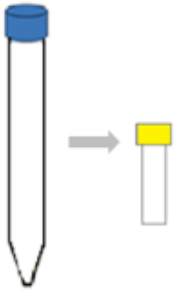
11. Enter collection data into the ATRI Data Portal website on day of visit.

CSF Preparation (15-20 ml total)

Step One

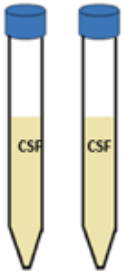
- Label tubes with pre-printed subject labels prior to collection.
- Pre-chill all cryovials on wet ice.

Step Two



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

Step Three

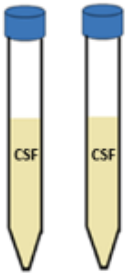


- Collect 10 - 20 ml total, including the 1-2 ml sent to the local lab.
- Collect sample into 2 15 ml conical tubes.

Step Four

- Place samples upright on wet ice until centrifugation begins.

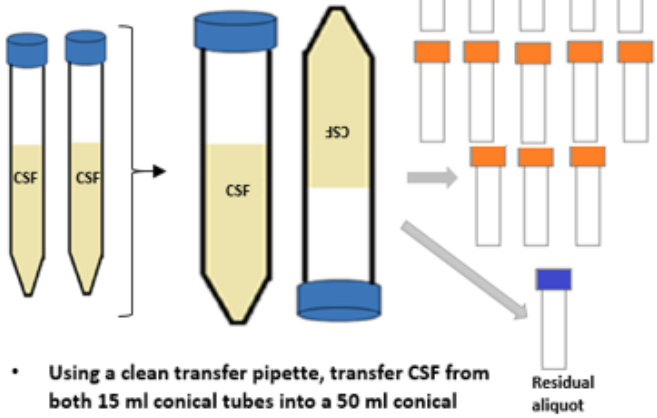
Step Five



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

Step Six

Step Seven



- Using a clean transfer pipette, transfer CSF from both 15 ml conical tubes into a 50 ml conical tube, leaving the debris in the bottom.
- Gently invert the 50 ml conical tube 3-4 times to mix the sample.
- Aliquot 1.5 ml into the orange-cap cryovials.
- If a residual aliquot is created, aliquot into blue-cap cryovial. Document specimen number and volume on CSF Sample Notification Form.
- Within 2 hours of CSF collection, samples need to be spun, aliquoted and in the freezer. Store at -80°C until shipment. Record time of freezing on CSF Sample Notification Form.

WELLNESS CHECK PHONE CALL

The Wellness Check to assess for side effects should occur according to the protocol. See protocol section 7.1.

SUGGESTED MANAGEMENT OF POST-LUMBAR PUNCTURE HEADACHE

Classic post-lumbar puncture (low pressure) headache is worse when the participant is upright (sits or stands) and improves when the participant is recumbent with the head **no higher** than the spinal cord.

Safety and comfort of the iLEADS LP is maximized by the use of atraumatic needles. The iLEADS protocol requires use of a 24-gauge Sprotte needle. Lumbar puncture is a standard procedure for collection of CSF but may be associated with pain during the performance of the procedure, comparable to the level of pain experienced during a blood draw. This is usually temporary and confined to the lower back. A persistent low-pressure headache may develop after lumbar puncture, probably due to leakage of CSF. If a post-LP headache persists it may need additional treatment, e.g. with fluids and analgesics. Uncommonly, a blood patch (injection of some of the participant's blood to patch the CSF leak) may be needed.

Prevention: Use of a small and atraumatic needle with careful technique are helpful in preventing lumbar puncture headache. Having the participant refrain from exercise or strenuous activities (especially heavy lifting) for 24 hours after the LP may minimize the chance of a lumbar puncture headache.

Treatment of headache after a lumbar puncture:

- Limit physical activity as much as possible for at least 24 hours post-procedure.
- Increase oral fluid intake. Caffeine may be helpful.
- Routine analgesics such as acetaminophen may be used.

Post-lumbar puncture headache often resolves with the above treatment. If the headache persists after 24 hours of this management, it will likely require a blood patch. A blood patch *typically* relieves the headache instantly.

Prior approval from the ATRI Coordinating Center is not necessary to perform a blood patch. Participants will be responsible for costs related to the performance of a blood patch.

9.0 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 a NCRAD Coordinator to alert them of the challenging blood draw or circumstances as to why biofluids were not collected.
    Schedule participant for a re-draw visit as quickly as possible.
    If the biofluids are still not collected successfully, track it as a protocol deviation.

9.1 Re-Draw Instructions and Timeframes

Sample Collection-Blood eCRF is a log form. Select '*Add a new record*' to enter a record. Enter one record per Date of Collection and specify samples collected. At least one sample type must be marked as collected on this date to successfully submit the form.

If a re-draw is necessary and occurs BETWEEN TWO VISITS, add a new record in the previous visit, making sure to include the re-draw Date of collection and Kit Number. If a sample was missed during a regularly scheduled visit, but a sample was collected PRIOR to NEXT scheduled visit, enter in the EDC as a re-draw. Also, provide reason for re-draw in the Comments section.

10.0 Packaging and Shipping Instructions

ALL study personnel responsible for shipping should be certified in biofluid shipping (i.e. IATA certification). The iLEADS Clinical Monitor will review training and certification through the study. If not available at your institution, please contact NCRAD with questions and information regarding resources.

Sample Type	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood (Plain Red-Top Serum Tube) for isolation of serum	1.5 ml serum aliquots per 2.0 ml cryovial (red cap); residual volume placed in 2.0 ml cryovial with blue cap	Up to 4	Frozen
Whole blood (10 ml Lavender-Top EDTA) for isolation of plasma & buffy coat (for DNA extraction)	1.5 ml plasma aliquots per 2.0 ml cryovial (lavender cap); residual volume placed in 2.0 ml cryovial with blue cap	Up to 10	Frozen
	1 ml buffy coat aliquot per 2.0 ml cryovial (clear cap)	3	Frozen
Whole blood (6 ml Lavender-Top EDTA) for CLIA lab testing	N/A	1	Frozen
Whole blood (3 ml Lavender-Top EDTA) for long read sequencing	1 ml whole blood aliquot per 2.0 ml cryovial (green cap)	3	Frozen
Whole blood for RNA extraction	N/A	1	Frozen
Whole blood for PBMC **optional*	N/A	N/A	N/A – Kept locally at site
CSF Collection **optional*	1.5 ml CSF aliquots per 2.0 ml cryovial (orange cap); residual volume placed in 2.0 ml cryovial with blue cap; 1-2 ml for local lab placed in 2.0 ml cryovial with yellow cap.	Up to 14	Frozen

10.1 Frozen Packaging Instructions

Sites will ship samples back to NCRAD using the applicable courier listed below.

Site	Courier
Lund	World Courier
Sant Pau-Barcelona	World Courier
Fleni-Argentina	World Courier
UCL	World Courier
Vumc – Amsterdam	SGS

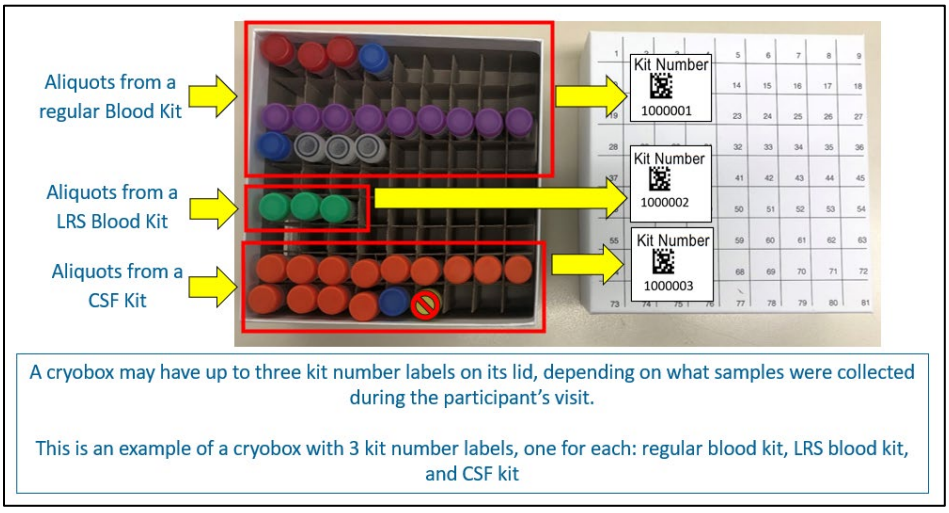
You should ship back to NCRAD when you have accumulated samples from 4 participant visits, or every six months, whichever is sooner. When you are ready to ship, contact the NCRAD coordinator (agericks@iu.edu) if using World Courier. If you are using SGS, contact them directly and prepare your shipment.

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.

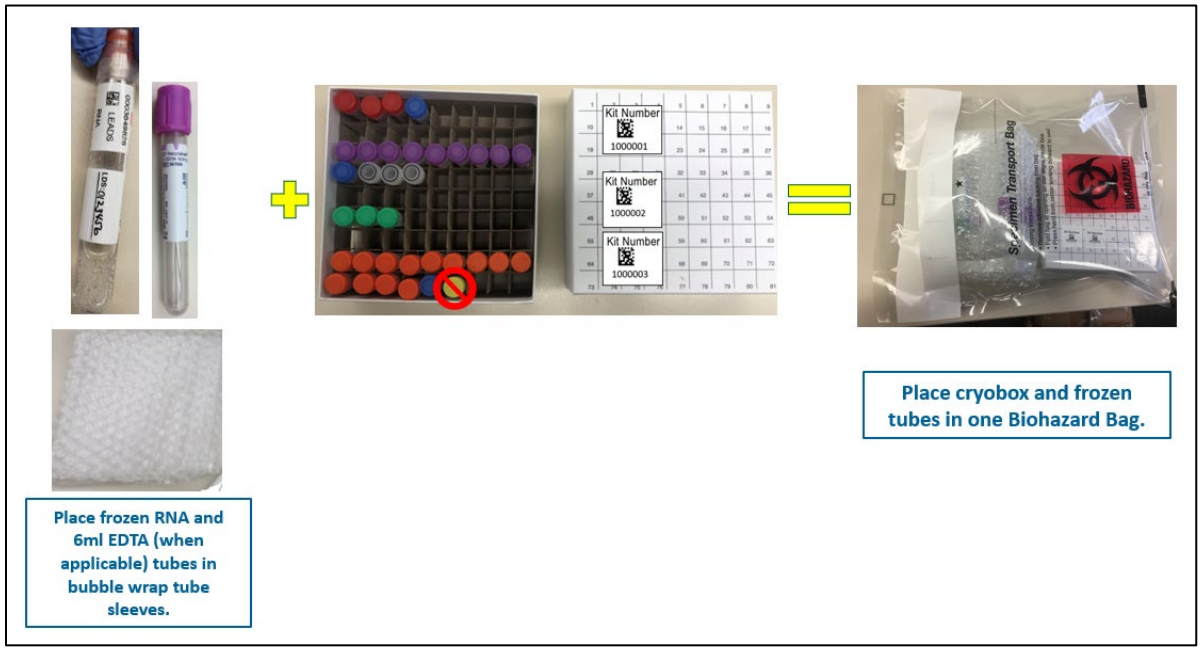
10.2 Instructions for Biohazard Bag Preparation

Samples should be placed in a biohazard bag. The biohazard bag will then be placed in a Styrofoam container for shipping.

1. Place all frozen labeled aliquots of serum, plasma, buffy coat, whole blood, and CSF aliquots from the same participant visit in the cryovial cryobox.
    a. Each 81-slot cryobox will hold approximately 34 cryovial samples. Place plasma, buffy coat, serum, whole blood (when applicable), and CSF aliquots within one cryobox. (4 serum, 10 plasma, 3 buffy coat, 3 whole blood (when applicable), and 14 CSF) per participant blood draw and CSF draw (see below).
    b. Cryoboxes should contain all of the specimens from the same patient, per time point.
2. Label the outside of the cryoboxes with the appropriate kit number label(s). There are up to three kit labels used per cryobox, one for blood components, one for long read sequencing components, and one for CSF. Place serum, plasma, buffy coat, whole blood, and CSF aliquots within one cryobox and place within a biohazard bag. The biohazard bags are large enough to contain one cryobox and up to 2 frozen blood tubes from one participant's visit.
    a. Remember: whole blood for long read sequencing will only be collected once over the entire course of a participant's participation in LEADS.
    b. Remember: whole blood for CLIA genetic testing will only be collected once over the entire course of a participant's participation in LEADS.



3. Place the cryobox in the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag). Place frozen 6ml EDTA tube and PAXgene™ tube in provided bubble wrap tube sleeves, seal, and place in the same biohazard bag. Seal biohazard bag according to the instructions on the bag.



10.3 Instructions for Shipping with World Courier

When you are ready to ship samples back to NCRAD, contact the NCRAD coordinator (agericks@iu.edu). NCRAD will work with your site and World Courier to arrange a shipment. Prior to pick up, please keep samples stored in a -80°C freezer.

There will be a waiting period between when you contact NCRAD to instigate a shipment and when the shipment occurs. This waiting period is expected to be up to 7 business days. For this reason, you should contact NCRAD about shipment approximately two weeks before you intend to ship.

World Courier will arrive with all of the supplies necessary for packaging and shipping the samples. Please note that World Courier will not handle unpackaged samples. Therefore, a site coordinator will need to place the samples into the packaging provided by World Courier. **It is imperative that a site coordinator is available to assist World Courier when they arrive**.

10.4 Instructions for Shipping with SGS

The Amsterdam site has chosen to coordinate shipping samples back to NCRAD via SGS. Amsterdam should obtain all shipping supplies through SGS. The most important issue for shipping is to maintain the temperature of the samples. The frozen samples must never thaw; not even the outside of the tubes should be allowed to defrost. This is best accomplished by making sure the Styrofoam container is filled completely with pelleted dry ice.

IMPORTANT
Frozen samples **MUST** be delivered to NCRAD Monday-Friday 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 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
- Dry Ice label, and net weight of dry ice contained

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.

FROZEN SAMPLES MUST BE DELIVERED TO NCRAD MONDAY - FRIDAY 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 Forms (Appendix B and Appendix C, include a copy in your shipment AND notify the NCRAD Study Coordinator by email at alzstudy@iu.edu (include tracking number in email) IN ADVANCE to confirm the shipment.

11.0 Data Queries and Sample Reconciliation

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

The Alzheimer's Therapeutic Research Institute (ATRI) data collection team and/or iLEADS Clinical Monitors will be collaborating with NCRAD to reconcile information captured in the database compared to samples received and logged at NCRAD. Information that appears incorrect in the ATRI 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.

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 6 months at the site.
- Use of an incorrect Biological or CSF Sample and Shipment Notification Form

12.0 Appendices List

Appendix A

Rate of Centrifuge Worksheet

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

Submitter Information

Name: **Site:**

Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

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

Calculating RPM from G-Force:

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

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

Comments:

Please send this form to NCRAD Study Coordinator

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

Appendix B

iLEADS International Longitudinal Early-Onset Alzheimer's Disease Study

Participant ID: LDS _ _ _ _ _ _ _ _

Biological Sample and Shipment Notification Form

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

NCRAD

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

General Information:

From: _____

Phone: _____

Email: _____

Date: _____

Study: iLEADS: ☐ CI Participant ☐ CN Participant

Visit (circle one): BASELINE M12 M24 M36 M48 M60 M72

Sex: ☐ M ☐ F

Year of Birth: _____

Tracking #: _____

Kit #:

KIT BARCODE

Kit # (Only if 3ml EDTA tube used for LRS):

KIT BARCODE

Blood Collection:

1. Date Drawn (MM/DD/YYYY): _____
2. Time of Drawn (24 hour clock): _____ [HHMM]
3. Last time subject ate (MM/DD/YYYY): _____
4. Last time subject at (24 hour clock): _____ [HHMM]

Blood Processing:

RNA (PAXgene Tube)

Total Volume of blood drawn (1 x 2.5 mL PAXgene RNA tube): _____ mL

Time PAXgene RNA tube placed in freezer (24 hour clock): _____ [HHMM]

Storage temperature of freezer: _____ °C

Serum (Red 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 (1x10 mL Serum tube): _____ mL

Original volume drawn (1x10 mL Serum tube): _____ mL

Time aliquoted: _____ [HHMM]

Number of 1.5 mL serum aliquots created: _____

If applicable, volume of residual serum aliquot (less than 1.5 mL-Blue cap): _____ mL

If applicable, specimen number of residual serum aliquot (Last four digits): _____

Time aliquots placed in freezer (24 hour clock): _____ [HHMM]

Storage temperature of freezer: _____ °C

EDTA (Lavender Top Tube – 3mL)

3mL EDTA tube for LRS collected? ☐ Yes ☐ No

Original volume drawn (1x3mL EDTA tube): _____ mL

Time aliquoted: _____ [HHMM]

Whole blood aliquot #1 (last four digits): _____

Whole blood volume #1: _____ mL

Whole blood aliquot #2 (last four digits): _____

Whole blood volume #2: _____ mL

Whole blood aliquot #3 (last four digits): _____

Whole blood volume #3: _____ mL

Whole blood aliquot #4 (last four digits): _____

Whole blood volume #4: _____ mL

Plasma & Buffy Coat (Lavender Top Tube – 10mL)

Time spin started (24 hour clock): _____

Duration of centrifuge: [HHMM]

Temp of centrifuge: _____ °C

Rate of centrifuge: _____ xg

Original volume drawn (3x10 mL EDTA tubes):

EDTA #1: _____ mL EDTA #2: _____ mL EDTA #3: _____ mL

Time aliquoted: [HHMM]

Number of 1.5 mL plasma aliquots created: _____

If applicable, volume of residual plasma aliquot (less than 1.5 mL-Blue cap): _____ mL

If applicable, specimen number of residual plasma aliquot (Last four digits): _____

Time aliquots placed in freezer (24 hour clock): _____ [HHMM]

Storage temperature of freezer: _____ °C

Buffy coat aliquot #1 (last four digits): _____

Buffy coat volume #1: _____ mL

Buffy coat aliquot #2 (last four digits): _____

Buffy coat volume #2: _____ mL

Buffy coat aliquot #3 (last four digits): _____

Buffy coat volume #3: _____ mL

Buffy coat aliquot #4 (last four digits): _____

Buffy coat volume #4: _____ mL

EDTA (Lavender Top Tube – 6mL)

6mL EDTA tube for CLIA testing collected? ☐ Yes ☐ No

Original volume drawn (1x6mL EDTA tube): _____ mL

Notes: _____

Version 3.2025

Appendix C

iLEADS international Longitudinal Early-Onset Alzheimer's Disease Study

Participant ID: LDS _ _ _ _ _ _ _

Biological 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	Phone: 1-800-526-2839

General Information:

From:_____

Phone: _____

Email: _____

Date:_____

Kit #:

KIT BARCODE

Study: iLEADS: ☐ CI Participant ☐ CN Participant

Visit (circle one): BASELINE M12 M24 M36 M48 M60 M72

Sex: ☐ M ☐ F

Year of Birth:_____

Tracking #:_____

CSF Collected? ☐ Yes ☐ No

Gauge needle used for LP: ☐ 22G ☐ 24G

CSF Collection:

1. Date of Collection (MM/DD/YYYY): _____
2. Time of Collection (24 hour clock): _____ [HHMM]
3. Last date subject ate (MM/DD/YYYY): _____
4. Last time subject at (24 hour clock): _____ [HHMM]

CSF Processing:

Total amount of CSF collected:	_____ mL
Time spin started (24 hour clock):	_____ [HHMM]
Duration of centrifuge:	_____ minutes
Temp of centrifuge:	_____ °C
Rate of centrifuge	_____ xg
Time aliquoted:	_____ [HHMM]
Number of 1.5mL aliquots created (up to 14 total): (Orange cap cryovial)	_____
If applicable, volume of CSF residual aliquot (Blue cap cryovial)	_____
If applicable, specimen number of residual CSF aliquot (Last four digits):	_____
Time aliquots placed in freezer (24 hour clock):	_____ [HHMM]
Storage temperature of freezer:	_____ °C

Notes:

_____

_____

_____

Version 3.2025