LIFE-DSR MANUAL OF PROCEDURES UPDATE:

V3.2023

Section	Change
Document Footer	The version date has been updated for this amendment.
Title Page	Title page updated to reflect version change.
Throughout document	1. Updated to reflect addition of 10 mL EDTA tubes. 2. Added instructions regarding batch shipping frozen plasma, CSF, and RNA samples together if collected same day as frozen main study shipment. 3. Added reminder to ensure all tubes are not expired prior to collection and processing of samples.
Section 3.4	Added Juneteenth
Section 5.2	Updated due to supply chain issues.
Section 6.1	Updated pictures of the Collection and Aliquot Labels that now have 2 barcodes.
Section 8.0	Included batch instructions for small shipping containers.
Section 8.2	Updated UPS Shipping information.
Section 12.2	Updated due to supply chain issues.
Section 13.1	Updated pictures of the Collection and Aliquot Labels that now have 2 barcodes.
Section 15.2	Clarified usage of refrigerant packs for ambient shipments.
Addendum 1	Added Part II – Subset – additional collection for subset (PBMC, RNA and CSF)
	1. Added section 13.2 Video List to link CSF Cell Isolation and Cryopreservation Demo Video 2. Made it clear that ALL Substudy specimens are to be shipped Monday – Wednesday ONLY on the same day of collection. 3. Updated CSF Pellet processing procedures to meet the needs of same day shipping: a. CSF is to be left in the CoolCell® at -80°C for at least 2 hours instead of 4 or overnight.
Last Page	MOP Approval Signature form on last page.

Longitudinal Investigation for Enhancing Down Syndrome Research (LIFE-DSR) Study

of the

Down Syndrome – Clinical Trials Network

in collaboration with

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

Biofluid Collection, Processing and Shipment

Manual of Procedures

Version 6.2

March 2023

Table of Contents

1.0 ABBREVIATIONS

AD	Alzheimer's Disease
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
DS-CTN	Down Syndrome Clinical Trials Network
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
LIFE-DSR	Longitudinal Investigation for Enhancing Down Syndrome Research
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
UPS	United Parcel Service

2.0 PURPOSE

The collection of biofluids is an important part of the Longitudinal Investigation for Enhancing Down Syndrome Research (LIFE-DSR) Study of the Down Syndrome – Clinical Trials Network (DS-CTN). 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 LIFE-DSR study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

The following samples will be sent to NCRAD:

- Plasma
- Buffy Coat (DNA Extraction)

***For a subset of participants, additional samples will be collected and sent to NCRAD:**

- ***RNA***
- ***PBMC***
- ***CSF***

Please see Addendum 1 for instructions for collection of these sample types.

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

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

3.0 NCRAD INFORMATION

3.1 NCRAD Contacts

Tatiana Foroud, PhD, NCRAD Principal Investigator
Phone: 317-274-2218

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

Colleen Mitchell, Laboratory Manager
Phone: 317-278-9016
Email: mitchecm@iu.edu

Zoë Potter, BA, CCRP, Study Coordinator
Phone: (317) 278-9086
Email: zdpotter@iu.edu

General NCRAD Contact Information
Phone: 1-800-526-2839
Email: alzstudy@iu.edu
Website: www.ncrad.org
LIFE-DSR Study Specific Webpage: https://ncrad.org/resource_life-dsr.html

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

3.2 Hours of Operation

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

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

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

3.3 Holiday Schedules

- Please note that courier services may observe a different set of holidays. Please be sure to verify shipping dates with your courier prior to any holiday.

3.4 Holiday Observations

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

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

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

- 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.
- **Although rarely occurs, weekend/holiday delivery must be arranged in advance with NCRAD staff.**

4.0 NCRAD LABORATORY COLLECTION

4.1 Site Required Equipment

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

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

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

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

In order to ship specimens, you must provide:
Dry ice (approximately 45 lbs. per shipment)

4.2 Biofluid Collection Schedules

LIFE-DSR Collection Schedule:

	Baseline	16M	32M
Plasma	X	X	X
DNA	X	X	X

Whole blood is collected in one type of tube (10 mL lavender-top EDTA tube). The 10 mL EDTA tubes are processed locally into plasma and buffy coat fractions; then aliquoted, frozen at the study site, and shipped to NCRAD.

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

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

4.3 BIOFLUID COLLECTION CHARTS

Biofluid Collection for Baseline, 16M, and 32M Visits

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	**EDTA (Lavender-Top) Blood Collection Tube (10 mL)**	**5**	**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)	17	1.5 mL plasma aliquot per 2.0 mL cryovial (lavender cap)	Up to 17	Frozen
	BUFFY COAT: 2.0 mL cryovial	5	1 mL buffy coat aliquot per 2.0 mL cryovial (clear cap)	5	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**).** Submit a copy to NCRAD with a reason provided for the omission and track it as a protocol deviation.

5.0 SPECIMEN COLLECTION KITS, SHIPPING KITS, AND SUPPLIES

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD, the Blood Supplemental Supply Kit, the Frozen Shipment Supply Kit 2) clinical lab supplies (with the exception of dry ice and equipment supplies listed in Section 4.1). The provided materials include blood tubes, pipettes, boxes for plasma/buffy coat aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, LIFE-DSR ID Labels, Collection and Aliquot Tube Labels will all be provided by NCRAD. Details regarding the blood kits are found in this Manual of Procedures. Collection and Aliquot Tube Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 6.1.

5.1 Specimen Collection Kit Contents

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

LIFE-DSR Blood-Based Kits

Quantity	Blood-Based Kit Components
5	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
16	Cryovial tube (2.0 mL) with lavender cap
1	Cryovial tube (2.0 mL) with blue cap
5	Cryovial tube (2.0 mL) with clear cap
2	Disposable graduated transfer pipette (3 mL)
1	50 mL conical (unwrapped)
27	Pre-printed Collection and Aliquot Tube Label
3	Pre-printed Kit Number Label
6	Labels for handwritten LIFE-DSR ID
1	Cryovial box (holds up to 25 cryovials)
1	Resealable bag
5	Resealable bubble wrap tube pouches

Blood-Based Supplemental Supply Kit

Quantity	Blood-Based Supplemental Supply Kit Components
15	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
25	Cryovial tube (2.0 mL) with lavender cap
5	Cryovial tube (2.0 mL) with blue cap
15	Cryovial tube (2.0 mL) with clear cap
5	Disposable graduated transfer pipette
5	50 mL conical (unwrapped)
10	Labels for handwritten LIFE-DSR ID
5	Cryovial box (holds up to 25 cryovials)

LIFE-DSR Frozen Blood Shipping Supply Kit

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

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module.
By Request	Cryovial box (holds up to 25 cryovials)
By Request	Cryovial tube (2.0 mL) with lavender cap
By Request	Cryovial tube (2.0 mL) with clear cap
By Request	Cryovial tube (2.0 mL) with blue cap
By Request	Shipping container for dry ice shipment

	(shipping and Styrofoam box)
By Request	Styrofoam shipping containers (11”x9”x8” 1 1/2” wall)
By Request	Plastic biohazard bag with absorbent sheet (small)
By Request	Disposable graduated transfer pipette
By Request	50 mL conical (unwrapped)
By Request	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
By Request	Warning label packet
By Request	UN3373 label
By Request	Biohazard label
By Request	UPS Dry ice shipping label
By Request	Fine point permanent markers
By Request	LIFE-DSR ID Labels

5.2 Kit Supply to Study Sites

Each site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits. Please go to: http://kits.iu.edu/lifedsr to request additional kits and follow the prompts to request the desired supplies. Options include ordering a specific number of kits; we are also including the option of simply ordering the desired amount of extra supplies.

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

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

6.0 BLOOD COLLECTION AND PROCESSING PROCEDURES

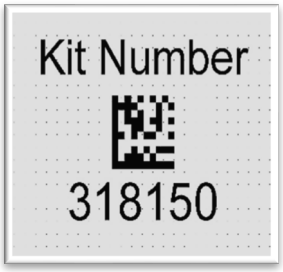
*****Important Note*****

In order to ensure the highest quality samples are collected, processed, and stored, it is essential to follow the specific collection, processing, and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood. Please note that the centrifuge may take 30 minutes to cool, so please plan accordingly.

6.1 Labeling Samples

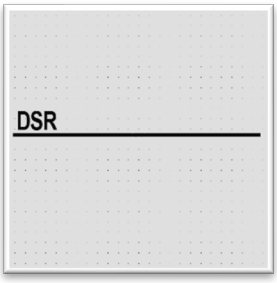
Label Type Summary

1. Kit Number Label
2. Collection and Aliquot Tube Label
3. LIFE-DSR ID Label



The **Kit Number Labels** do not indicate a specimen type but are affixed on the Biological Sample and Shipment Notification Form and on specific packing materials. This label ties together all specimens collected from one subject at one visit.

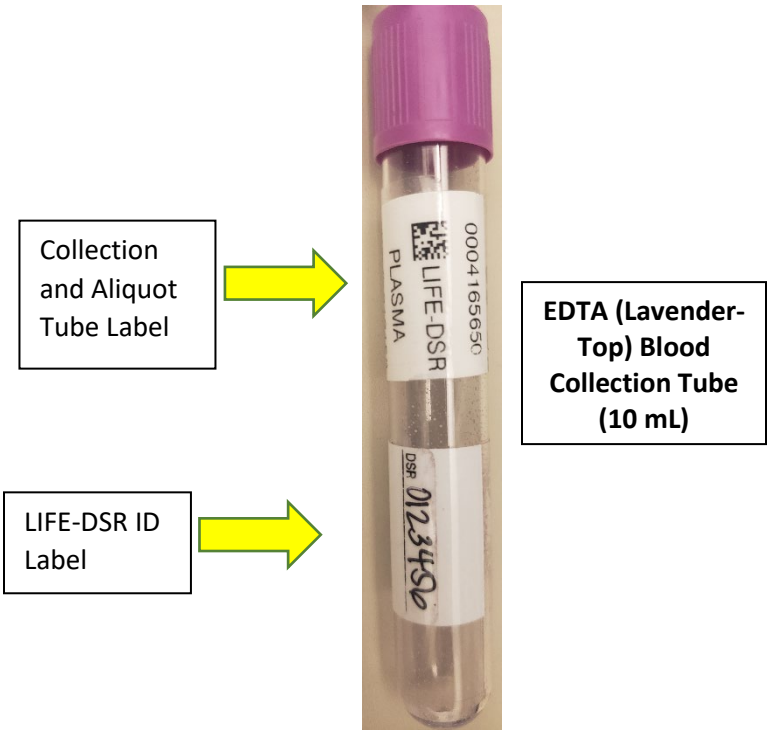
The **Collection and Aliquot Tube Labels** for blood derivatives are placed on all collection and aliquot tubes.



The **LIFE-DSR ID Labels** are placed on all collection tubes. This label is used to document the individual's unique LIFE-DSR ID.

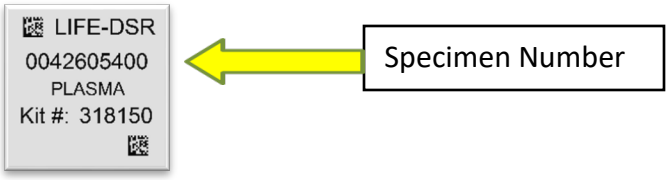
Important Note

Each collection tube will contain two labels: The Collection and Aliquot Tube Label and the LIFE-DSR 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 LIFE-DSR ID label below.



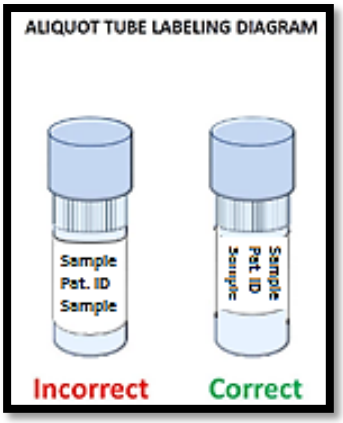
In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- 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 near 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 the LIFE-DSR ID Labels and place on the collection tubes only (EDTA) **BEFORE** sample collection, processing, or freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place LIFE-DSR ID labels on any cryovials.
- The Collection Tube Labels contain 2D barcodes on the top left-hand and bottom right-hand side of the label. Place 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 each tube. It may be helpful to roll the tube between your fingers after applying the label.
- If there are any unused cryovials, **please do not send the empty cryovials to NCRAD**.



These unused cryovials (ensure labels are removed) can be saved as part of a supplemental supply at your site or the cryovials can be disposed of per your site's requirements.

6.2 Video List

➢ The following training videos are available to assist you with the specimen processing, aliquoting, and shipping processes. The videos are available at:

NCRAD - LIFE-DSR Active Study Page

- LIFE-DSR MOP Training
- Plasma and Buffy Coat Processing and Aliquoting
- Frozen Shipping

6.3 Filling Aliquot Tubes (Plasma)

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

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

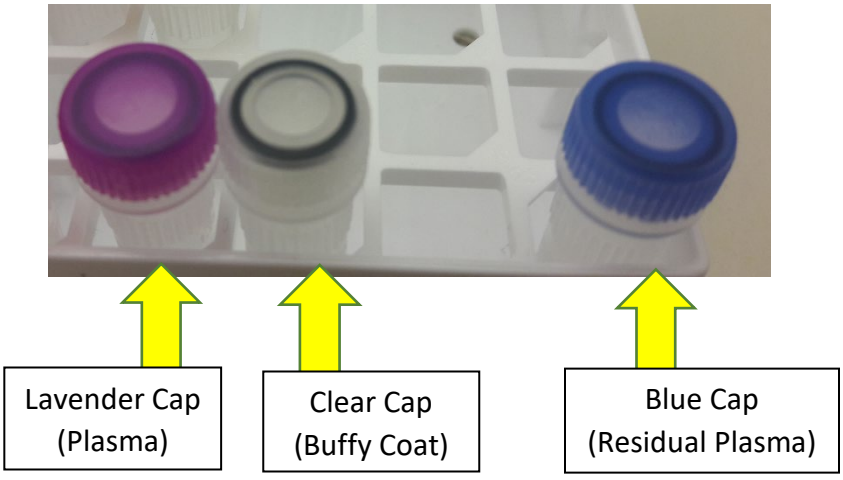
Aliquot the remaining biological material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 3.6 mL of sample

is obtained, you should fill 2 cryovial tubes each with 1.5 mL, and one additional cryovial tube with the remaining 0.6 mL.

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). 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
Lavender Cap	Plasma
Clear Cap	Buffy Coat
Blue Cap	Residual (plasma)



6.4 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 aliquots)

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 LIFE-DSR ID Label and pre-printed "**PLASMA**" Collection and Aliquot Tube Label on the lavender-top EDTA tubes. Place pre-printed "**PLASMA**" Collection and Aliquot Tube Labels on the (16) 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 "**BUFFY COAT**" Collection and Aliquot Tube Label on the (5) 2.0 mL cryovial with a clear lid.

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

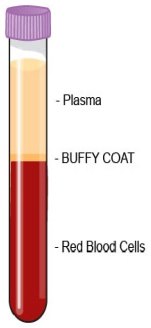
5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 mL of blood into the tube.

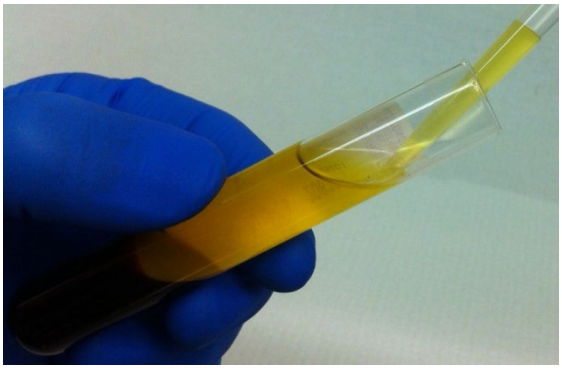
   If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.

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

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

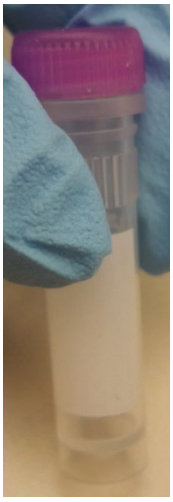
8. Centrifuge balanced tubes for 10 minutes at 2000 x g and 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM.)**
   a. Equivalent rpm for spin at 2000 x g
   b. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
   c. Record original volume drawn for each tube in spaces provided on the Biological Sample Shipment and Notification Form.
   d. Plasma samples need to be spun, aliquoted, and placed in the freezer within 1 hour from the time of collection.
   e. 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 five EDTA tubes into the 50 mL conical tube and gently invert 3 times. Aliquot 1.5 mL per cryovial (total vials = up to 17 with 1.5 mL each). Each 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 (17 possible) and 5 Buffy Coat Aliquots

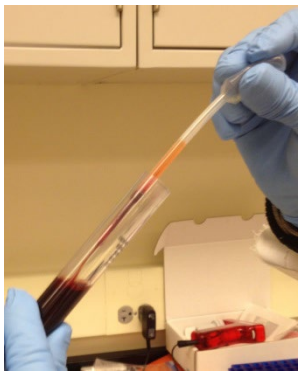


Close up view of 2.0 ml Cryovial

10. Place the labeled cryovials in the 25 cryovial box and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample 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 caps 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.

Buffy Coat layer (mixed with RBCs)



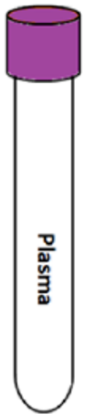
Buffy Coat Aliquot (Please use CLEAR CAP

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 25 cryovial box 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.

Plasma and Buffy Coat Preparation (10ml Lavender-Top Tube x 5)

Step One

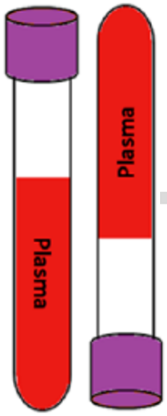


- Store tubes at room temperature.
- Label tubes with preprinted labels prior to blood draw.

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.

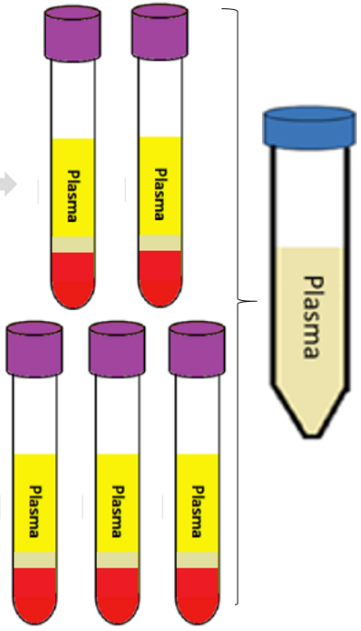
Step Four

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

Step Five

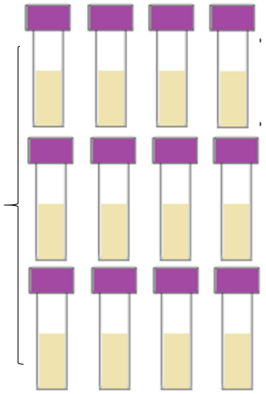
- Centrifuge samples at 2000 x g at 4°C for 10 minutes.
- Samples need to be spun, aliquoted, and in the freezer within 1 hour from the time of collection.

Step Six



- Pool all plasma from the 5 EDTA tubes into a 50ml conical tube and invert gently 3 times to mix the plasma.

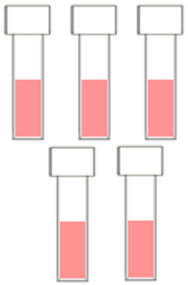
Step Seven



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

7.0 INCOMPLETE OR DIFFICULT BLOOD DRAWS

*****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.

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

1. If the biofluids at a scheduled visit **are partially** collected:
    a. Attempt to process and submit any samples that were able to be collected during the visit.
    b. Document difficulties on the 'Biological Sample and Shipment Notification Form' prior to submission to NCRAD.
        i. Indicate blood draw difficulties at the bottom of the 'Biological Sample and Shipment Notification Form' within the "Notes" section.
        ii. Complete the 'Biological Sample and Shipment Notification Form' with tube volume approximations and number of aliquots created.
    c. Contact a NCRAD coordinator and alert them of the challenging blood draw.

2. If the biofluids at a scheduled visit **are not** collected:
    a. Contact the LIFE-DSR Study Team to alert them of the challenging blood draw or circumstances as to why biofluids were not collected:
        a. Aisha Vanderhorst: avanderhorst@lumindidsc.org
        b. Jill MacDougall: jmacdougall@lumindidsc.org
        c. LuMind General Contact: lifedsr@lumindidsc.org

8.0 PACKAGING AND SHIPPING INSTRUCTIONS

ALL study personnel responsible for shipping should be certified in biofluid shipping (i.e., IATA certification). 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 (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 17	Frozen
	1 mL buffy coat aliquot per 2.0 mL cryovial (clear cap)	5	Frozen

8.1 Frozen Packaging Instructions

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 SHIPPED MONDAY-WEDNESDAY ONLY!

*****Important Note for Frozen Shipments ONLY*****

Batch shipping main study specimens and subset study specimens together: If shipping main study specimens same day as a subset collection, the 25-slot cryoboxes holding plasma and buffy coats from main study can be batch shipped with the subset specimens (PAXgene™ tube, 15 mL conical holding Cryogenic vial of CSF cells, and 48-slot cryobox holding CSF aliquots). Ensure there is ~45 lbs. of dry ice for large shippers and ~14 lbs. for small shippers.

Large Frozen Shipper – fits 5 25-slot cryoboxes and ~45 lbs. dry ice

Small Shipper – fits 3 25-slot cryoboxes and ~14 lbs. dry ice

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 and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the
- following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible Person
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373
  - ✓ UPS Dry Ice label and net weight of dry ice contained

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 Packaging Instructions

1. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu
   Attach the following to the email:
   a. Completed Biological Sample and Shipment Notification Form to the email notification.
   (See Appendix B for an example of the NCRAD sample form)

b. If email is unavailable, please call NCRAD (800-526-2839) and do not ship until you have contacted and notified NCRAD coordinators about the shipment in advance.

2. Place all frozen labeled aliquots of plasma and buffy coat aliquots from the same subject in the cryovial cryobox.

   a. Each 25-slot cryobox will hold approximately 22 cryovial samples. Place plasma and buffy coat within one cryobox (17 plasma, 5 buffy coat) per participant blood draw (see below).

One cryobox containing plasma, residual, and buffy coat aliquots.

   b. Cryoboxes should contain all of the specimens from the same patient, per time point.
   c. Batch shipping should be performed every (3) three months or when specimens from 5 participants accumulates, whichever is sooner.
   d. If small shipper, batch ship up to 3 participants.

*****Important Note for Frozen Shipments ONLY*****

Batch shipping main study specimens and subset study specimens together: If shipping main study specimens same day as a subset collection, the 25-slot cryoboxes holding plasma and buffy coats from main study can be batch shipped with the subset specimens (PAXgene™ tube, 15 mL conical holding Cryogenic vial of CSF cells, and 48-slot cryobox holding CSF aliquots). ***Ensure there is ~45 lbs. of dry ice for large shippers and ~14 lbs. for small shippers.***

3. Label the outside of the cryoboxes with the appropriate kit number label(s). Place plasma and buffy coat aliquots within one cryobox and place within a biohazard bag. The biohazard bags are large enough to contain one cryobox from one subject's visit.
4. Place the cryobox in the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag). Seal biohazard bag according to the instructions on the bag.

Place kit number label(s) on cryobox

5. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam shipping container.
6. Place the biohazard bag into the provided Styrofoam-lined shipping container on top of the dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container (as pictured below).
7. Fully cover the cryoboxes and tubes with approximately 2 inches of dry ice.
8. The inner Styrofoam shipping container must contain approximately 45 lbs. (or 21kg) of dry ice. The dry ice should entirely fill the inner box to ensure the frozen state of the specimens.

Full Shipping Container with Batched Samples and Dry Ice

Large Frozen Shipper – fits 5 25-slot cryoboxes and ~45 lbs. dry ice

Small Shipper – fits 3 25-slot cryoboxes and ~14 lbs. dry ice

9. Replace the lid on the Styrofoam carton. Place the completed Biological Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape.
   a. Attach provided UPS label for packages.
10. Complete the UPS Dry Ice Label
    a. Net weight of dry ice in kg (must match amount on the airbill)

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

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

IMPORTANT!
Ensure UPS address label is attached, and UPS Dry Ice label is filled out, or UPS may reject or return your package.

12. Hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off.
13. Specimens should be sent to the below address via **UPS Next Day Air**. Frozen shipments should be sent **Monday through Wednesday** to avoid shipping delays on Thursday or Friday. UPS does not replenish dry ice if shipments are delayed or held over during the weekend.

LIFE-DSR at NCRAD
Indiana University School of Medicine
351 West 10th Street
TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

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

*****Important Note*****
For frozen shipments, include no more than five cryovial boxes (separated by patient within 5 biohazard bags) per shipping container 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 and buffy coat will be shipped to NCRAD as outlined above.

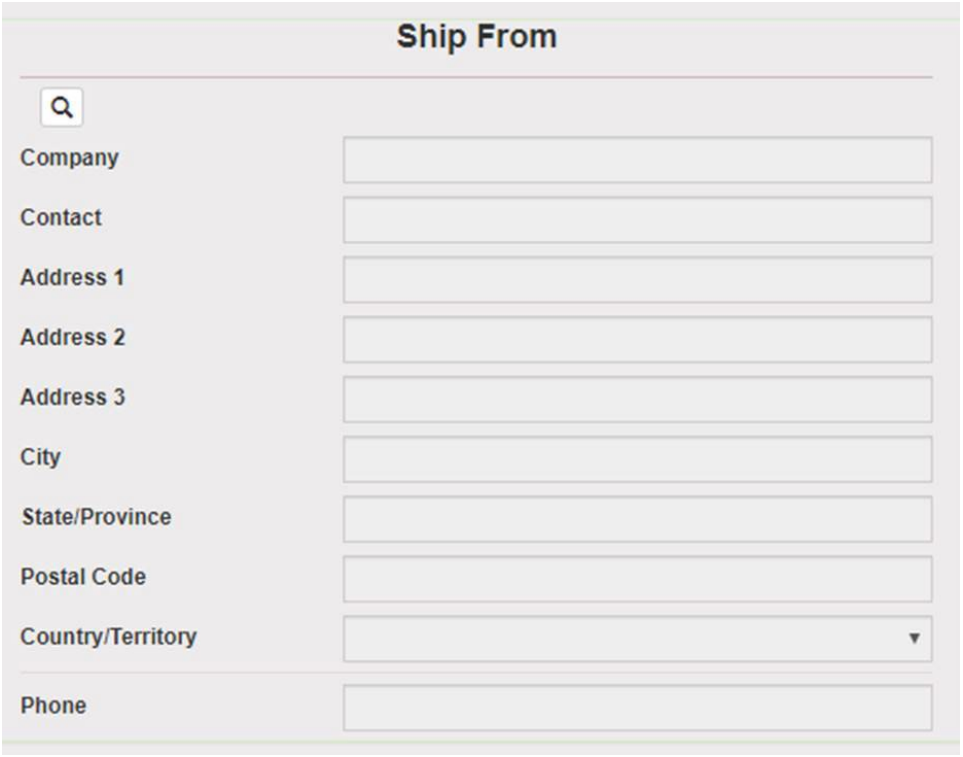
SHIP ALL FROZEN SAMPLES MONDAY - WEDNESDAY ONLY!
BE AWARE OF HOLIDAYS!!
BE AWARE OF INCLEMENT WEATHER THAT MAY DELAY SHIPMENT/DELIVERY OF SAMPLES

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

8.2 Frozen Shipping Instructions

1. Log into the ShipExec Thin Client at kits.iu.edu/UPS.
   a. If a new user or contact needs access, please reach out to your study contact for access.
2. Click “Shipping” at the top of the page and select “Shipping and Rating”

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



addresses within your study.
4. Click on the magnifying glass icon in the “Ship From” section to search for your shipping address.
   a. Search by Company (site), Contact (name), or Address 1 (first line of your site’s street address). Click Search.
   b. Click Select to the left of the correct contact information.
5. Verify that both the shipping information AND study reference are correct for this shipment.
   a. If wrong study contact or study reference, click Reset in the bottom right of the screen to research for the correct information.
6. Enter Package Information
   a. Ambient shipments
      i. Enter the total weight of your package in the “Weight” field and leave the “Dry Ice Weight” field empty.

   b. Frozen shipments
      i. Enter the total weight of your package in the “Weight” field.
      ii. Enter the dry ice weight in the “Dry Ice Weight” field.
      iii. If the “Dry Ice Weight” field is higher than the “Weight” field, you will receive an error message after clicking “Ship” and need to reenter these values.
   c. Click Ship in the bottom right of the page when complete.
7. If your site does not already have a daily UPS pickup, you will need to schedule one
   a. Click the blue Pickup Request button. Enter the earliest pickup time and latest pickup time in 24-hr format.
   b. Give a name & phone number of someone who the UPS driver can call if having issues finding the package.
   c. Give the Floor and Room Number (if needed) to be as descriptive as possible where this package needs to be picked up from. Click Save.
8. Print the airbill that is automatically downloaded.
   a. To reprint airbill, click History at the top left of the page.
   b. Click Detailed Report from the dropdown menu on the right side of the page.
   c. Enter tracking number if known. Otherwise, search by ship date. Click Search.
   d. Click print icon on right side of the tracking number line.
9. Fold airbill and place inside plastic UPS sleeve.
10. Peel the back off of the UPS sleeve and stick the sleeve to the package.
11. A UPS Pickup is automatically scheduled at the address you are shipping from, and the pickup is charged to NCRAD.
   a. If shipment occurs too late in the day for an automatic UPS pickup, you will receive an email stating that the pickup could not be scheduled, and you will need to make other arrangements.

9.0 DATA QUERIES AND SAMPLE RECONCILIATION

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

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 LIFE-DSR database.
- Samples that are frozen and stored longer than one quarter at the site
- Use of an incorrect Biological Sample and Shipment Notification Form

10.0 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

alzstudy@iu.edu

Appendix B

Participant ID: DSR __ __ __ __ __ __ __ __

Biological Sample and Shipment Notification Form

Please email or fax this from prior to the date of shipment.

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

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

From: _____ Date: _____

Phone: _____ Email: _____

Study: LIFE-DSR **Kit #:** KIT BARCODE

Visit (circle one): **BASELINE** **MONTH 16** **MONTH 32**

Sex: ☐ M ☐ F **Year of Birth:** _____

Blood Collection:

1. Date Drawn:	[MMDDYY]	2. Time of Draw:	[HHMM]
3. Last time subject ate:	[MMDDYY]	4. Last time subject ate:	[HHMM]

Blood Processing:

Plasma & Buffy Coat (Lavender-top) Tube (10 mL)		
Time spin started:		[HHMM]
Duration of centrifuge:		Minutes
Temp of Centrifuge: ____ °C Rate of centrifuge: ____ x g		
Time aliquoted:		[HHMM]
Number of 1.5 mL plasma aliquots created (lavender cap):		
If applicable, volume of residual plasma aliquot (less than 1.5 mL in blue cap):		mL
If applicable, specimen number of residual plasma aliquot (last four digits):		
Buffy coat #1 last four digits of specimen number:		
Buffy coat #1 volume: _____ mL	Original blood volume drawn: _____	mL
Buffy coat #2 last four digits of specimen number:		
Buffy coat #2 volume: _____ mL	Original blood volume drawn: _____	mL
Buffy coat #3 last four digits of specimen number:		
Buffy coat #3 volume: _____ mL	Original blood volume drawn: _____	mL
Buffy coat #4 last four digits of specimen number:		
Buffy coat #4 volume: _____ mL	Original blood volume drawn: _____	mL
Buffy coat #5 last four digits of specimen number:		
Buffy coat #5 volume: _____ mL	Original blood volume drawn: _____	mL
Time aliquots placed in freezer:		[HHMM]
Storage temperature in freezer:		°C

Notes:

Version (04.2021)

ADDENDUM 1 SUBSET – RNA, PBMC AND CSF

- This section provides collection, processing and shipment information for sites that are participating in the LIFE-DSR Substudy. The RNA, PBMC and CSF collection for subset of participants will be conducted on a different calendar day than the LIFE-DSR parent protocol study visit due to the combined total volume of blood.

11.0 RNA, PBMC AND CSF COLLECTION FOR SUBSET OF PARTICIPANTS

11.1 Site Required Equipment

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

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

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

- Centrifuge capable of ≥ 350 x g with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:
Dry ice (about approximately ~10 lbs. per shipment)

CoolCell® equipment will be provided by NCRAD as well.

11.2 BIOFLUID COLLECTION SCHEDULES

LIFE-DSR Subset Collection Schedule:

	Baseline/16M*	16M/32M*
RNA	X	X
PBMC	X	X
CSF	X	X

*Collection will be at 2 time-points – Either BL and M16 OR M16 and M32.

Whole blood is collected in two types of tubes (2.5 mL PAXgene™ tube, 10 mL green-top Sodium Heparin (NaHep) tube). The PAXgene™ tube is frozen locally without further processing. The Sodium Heparin tubes are shipped ambient to NCRAD on the day of the participant visit (Monday through Thursday only) without further processing.

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

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

11.3 BIOFLUID COLLECTION CHARTS

Subset Blood and CSF Biofluid Collection

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
Whole blood for RNA extraction	**PAXgene™ Blood Collection Tube (2.5 mL)**	**1**	**N/A**	**1**	Frozen
Whole blood for PBMC	**Sodium Heparin (Green-Top) Blood Collection tube (10 mL)**	**5**	**N/A**	**5**	Ambient
CSF Collection	**Wrapped Conical Tube (15 mL)**	**2**	**N/A**	**N/A**	**N/A**
	Cryogenic Vial – CSF pellet	1 cryovial (orange cap with ridges)	Up to 2 mL	1	Frozen
	Sterile Container – CSF supernatant	14 cryovial tubes (13 orange cap smooth, 1 blue cap)	1.5 mL CSF aliquots per 2.0 mL cryovial (orange cap smooth); residual volume placed in 2.0 mL cryovial with blue cap	Up to 14	Frozen
	Sterile Container	1 yellow cap cryovial tube	1-2 mL for local lab placed in 2.0 mL cryovial with yellow cap.	**0 – do not return to NCRAD**	N/A

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 D).** Submit a copy to NCRAD with a reason

provided for the omission and track it as a protocol deviation in the study's Electronic Data Capture (EDC) system.

12.0 SPECIMEN COLLECTION KITS, SHIPPING KITS AND SUPPLIES

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD, Ambient Shipping Supply kits and the Frozen Shipment Supply Kit 2) clinical lab supplies (with the exception of dry ice and equipment supplies listed in Section 11.1). The provided materials include blood tubes, pipettes, boxes for CSF aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, LIFE-DSR ID Labels, Cryovial Tube labels and Collection Tube Labels will all be provided by NCRAD. Details regarding the blood kits are found in this Manual of Procedures. Collection and Aliquot Tube Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 13.1.

12.1 Specimen Collection Kit Contents

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

Each site will also receive 2 CoolCell® containers, 24 CoolCell® Filler Vials and 9 cold aliquots (~1.6 mL) of Freeze Medium in a 25-cell cryobox. An additional 9 cold aliquots (~1.6 mL) of Freeze Medium in a 25-cell cryobox will be provided to sites in advance of the follow up visits.

***Important Note:** A Blood Substudy Collection Kit, CSF Substudy Collection Kit, and 22G LP Tray Kit must be ordered for each Substudy visit.*

LIFE-DSR Blood and CSF Substudy Kits

Quantity	Blood Substudy Collection Kit Components
1	PAXgene tube, 2.5 mL
5	NaHep tube, 10 mL
1	Resealable bag
5	Bubble wrap sleeve
3	Kit Number Labels
6	Collection Tube Labels
7	LIFE-DSR PT ID labels

Quantity	CSF Substudy Collection Kit Components
3	15 mL conical (individually wrapped)
1	50 mL conical tubes (individually wrapped)
1	2 mL Robotic Freezer Tubes - BLUE
1	2 mL Robotic Freezer Tubes - YELLOW
13	2 mL Robotic Freezer Tubes – ORANGE Smooth
1	2 mL self-standing Cryogenic Vial
5	3.0 mL disposable pipettes
1	Cryobox, 48 cell
1	Resealable bag
1	Bubble wrap sleeve
3	Kit Number Labels
1	Collection Tube Labels
14	Cryovial Labels
2	LIFE-DSR PT ID labels

Quantity	22G Lumbar Puncture Tray Kit Components
1	Sprotte needle, 22G X 3.5" (90mm)
1	Introducer needle, 1 mm x 30 mm
1	Hypodermic needle, 22G x 1.5"
1	Plastic syringe, (3 ml, Luer lock) with 25G x 5/8" needle attached
4	Polypropylene syringe (6 ml, Luer lock)
1	Needle stick pad
1	Adhesive bandage
1	Drape, fenestrated, 2 tabs, paper, 18" x 26"
2	Towel, 13.5" x 18"
6	Gauze pad, 2" x 2"
3	Sponge stick applicator
2	Lidocaine 1%, 5 ml
1	Povidone-Iodine Topical Solution, 0.75 oz

LIFE-DSR Substudy Ambient Blood Shipping Supply Kit

Quantity	Ambient Shipping Kit Components
1	Plastic biohazard bag with absorbent sheet
1	Small IATA shipping box with insulated cooler
1	Small refrigerant pack
1	Aqui-Pak 6 tube absorbent pouch
1	UN3373 Biological Substance Category B label
1	List of contents card
1	UPS return airbill pouch
1	Temperature monitor

LIFE-DSR Substudy Frozen Sample Shipping Supply Kit

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

LIFE-DSR Substudy Supplemental Biospecimen Kit

Quantity	Substudy Supplemental Kit Components
5	PAXgene tube, 2.5 mL
10	NaHep tube, 10 mL
5	15 mL conical (individually wrapped)
5	50 mL conical tubes (individually wrapped)
5	2 mL Robotic Freezer Tubes - BLUE
5	2 mL Robotic Freezer Tubes - YELLOW
20	2 mL Robotic Freezer Tubes – ORANGE Smooth
5	2 mL self-standing Cryogenic Vial
10	3.0 mL disposable pipettes
1	Resealable bag

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module.
By Request	PAXgene tube, 2.5 mL
By Request	NaHep tube, 10 mL
By Request	Cryovial box (holds up to 48 cryovials)
By Request	2 mL Robotic Freezer Tubes - BLUE
By Request	2 mL Robotic Freezer Tubes - YELLOW
By Request	2 mL Robotic Freezer Tubes – ORANGE Smooth
By Request	Cold aliquots (~1.6 mL) of Freeze Medium in a 25-cell cryobox
By Request	2 mL self-standing Cryogenic Vial
By Request	Shipping container for dry ice shipment (shipping and Styrofoam box)
By Request	Styrofoam shipping containers (11”x9”x8” 1 1/2” wall)
By Request	Plastic biohazard bag with absorbent sheet (large)
By Request	Disposable graduated transfer pipette
By Request	15 mL conical (individually wrapped)
By Request	50 mL conical (individually wrapped)
By Request	Warning label packet
By Request	UN3373 label
By Request	Biohazard label
By Request	UPS Dry ice shipping label
By Request	Fine point permanent markers
By Request	LIFE-DSR ID Labels

12.2 Kit Supply to Study Sites

Each site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits. Please go to: http://kits.iu.edu/lifedsr to request additional kits and follow the prompts to request the desired supplies. Options include ordering a specific number of kits; we are also including the option of simply ordering the desired amount of extra supplies.

Please allow **TWO - 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.

13.0 SAMPLE COLLECTION AND PROCESSING PROCEDURES

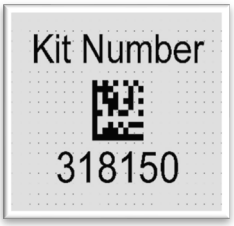
*****Important Note*****

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. Please note that the centrifuge may take 30 minutes to cool, so please plan accordingly.

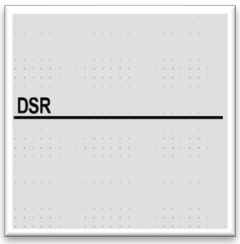
13.1 Labeling Samples

Label Type Summary

1. Kit Number Label
2. LIFE-DSR ID Label
3. Collection Tube Label
4. Cryovial Label



The **Kit Number Labels** do not indicate a specimen type but are affixed on the Biological Sample and Shipment Notification Form and on specific packing materials. This label ties together all specimens collected from one subject at one visit.



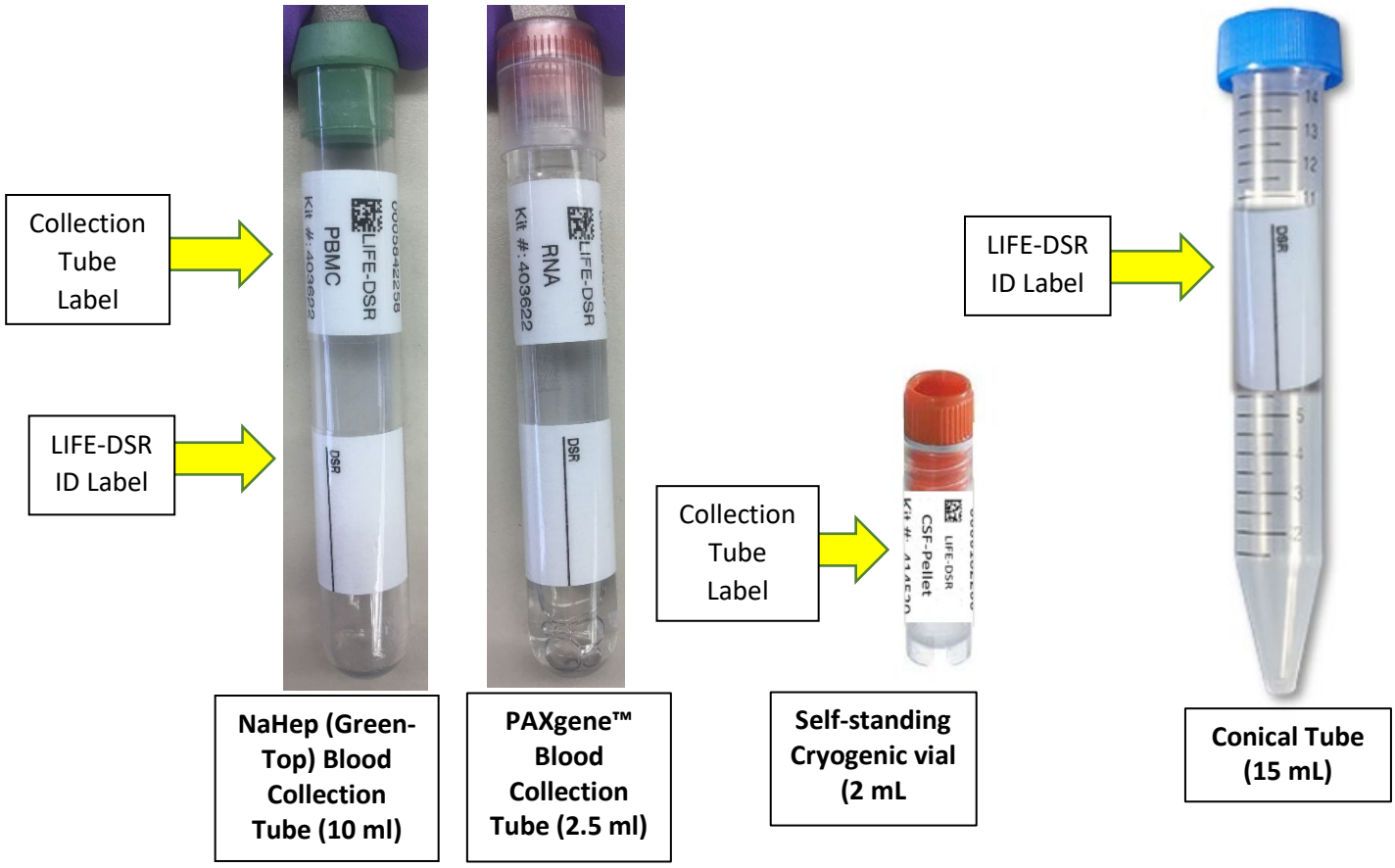
The **LIFE-DSR ID Labels** are placed on all collection tubes and 15 mL conical tubes. This label is used to document the individual's unique LIFE-DSR ID.

The **Collection Tube Labels** for blood derivatives are placed on all collection tubes and cryogenic vial (2 mL) containing CSF cells/pellet.

The small **Cryovial Labels** are placed on each CSF cryovial.

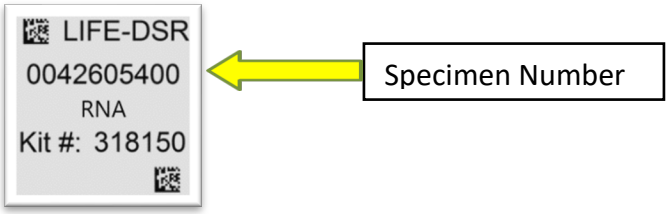
Important Note

Each collection tube will contain two labels: The Collection Tube Label and the LIFE-DSR 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 LIFE-DSR ID label below.



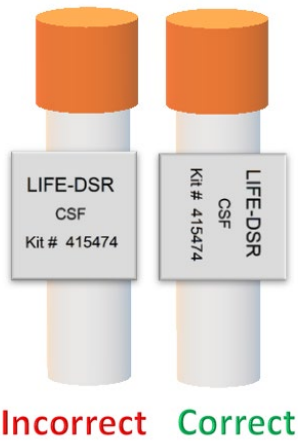
In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place labels on **ALL** 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.
- Using a fine point permanent marker, fill-in and place the LIFE-DSR ID Labels on the collection tubes only (NaHep and RNA) **BEFORE** sample collection, processing, or freezing. These labels are placed on collection tubes in addition to the Collection Tube Label.



- The Collection Tube Labels contain 2D barcodes on the top left-hand and bottom right-hand side of the label. Place this barcode toward the tube cap.

- Place Cryovial Labels **horizontally** on the CSF cryovials (wrapped around sideways if the tube is upright).



Cryovial Label Diagram

- Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label.

If there are any unused cryovials, please do not send the empty cryovials to NCRAD. These unused cryovials are specific to each kit and must disposed of per your site's requirements.

13.2 Video List

- The following training videos are available to assist you with the specimen processing, aliquoting, and shipping processes. The videos are available at: NCRAD - LIFE-DSR Active Study Page

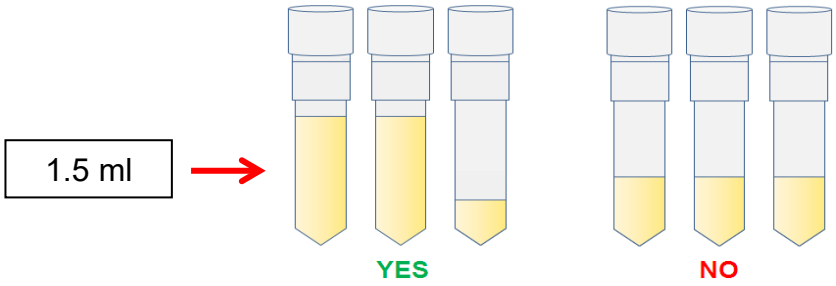
  - Frozen Shipping
  - Ambient Shipping – *Edit: Place cold pack in refrigerator, not the freezer. See Section 15.2.*
  - CSF Cell Isolation and Cryopreservation Demo Video

13.3 Filling Aliquot Tubes (CSF)

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

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

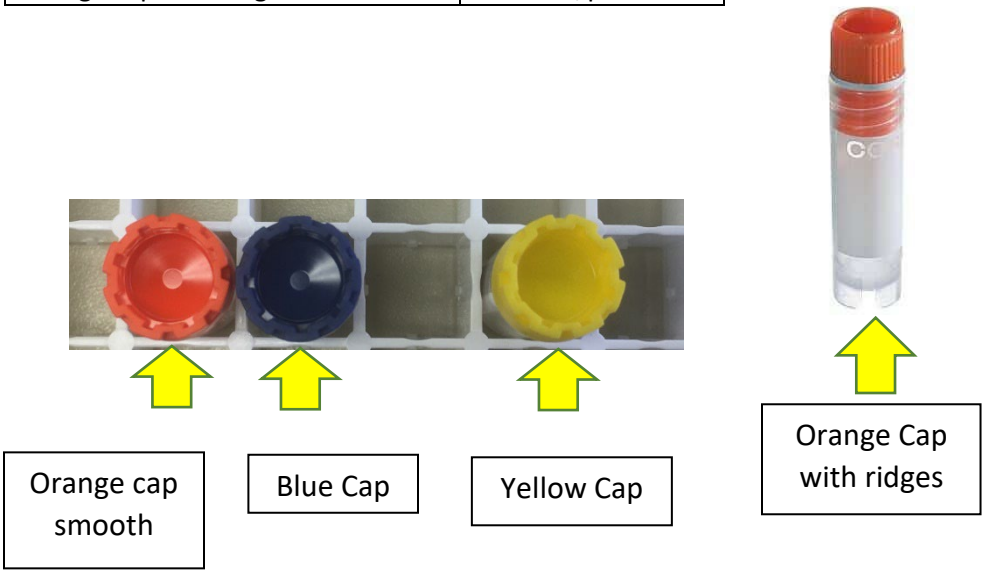
Aliquot the remaining biological material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 3.6 mL of sample is obtained, you should fill 2 cryovial tubes each with 1.5 mL, and one additional cryovial tube with the remaining 0.6 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). 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
Orange Cap smooth	CSF
Blue Cap	Residual
Yellow Cap	CSF for local lab
Orange cap with ridges	CSF cells/pellet



13.4 2.5 mL PAXgene™ Tube for RNA x 1 Whole Blood Collection for Isolation of RNA

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

1. Place filled-out LIFE-DSR ID Label and Collection "**RNA**" 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.

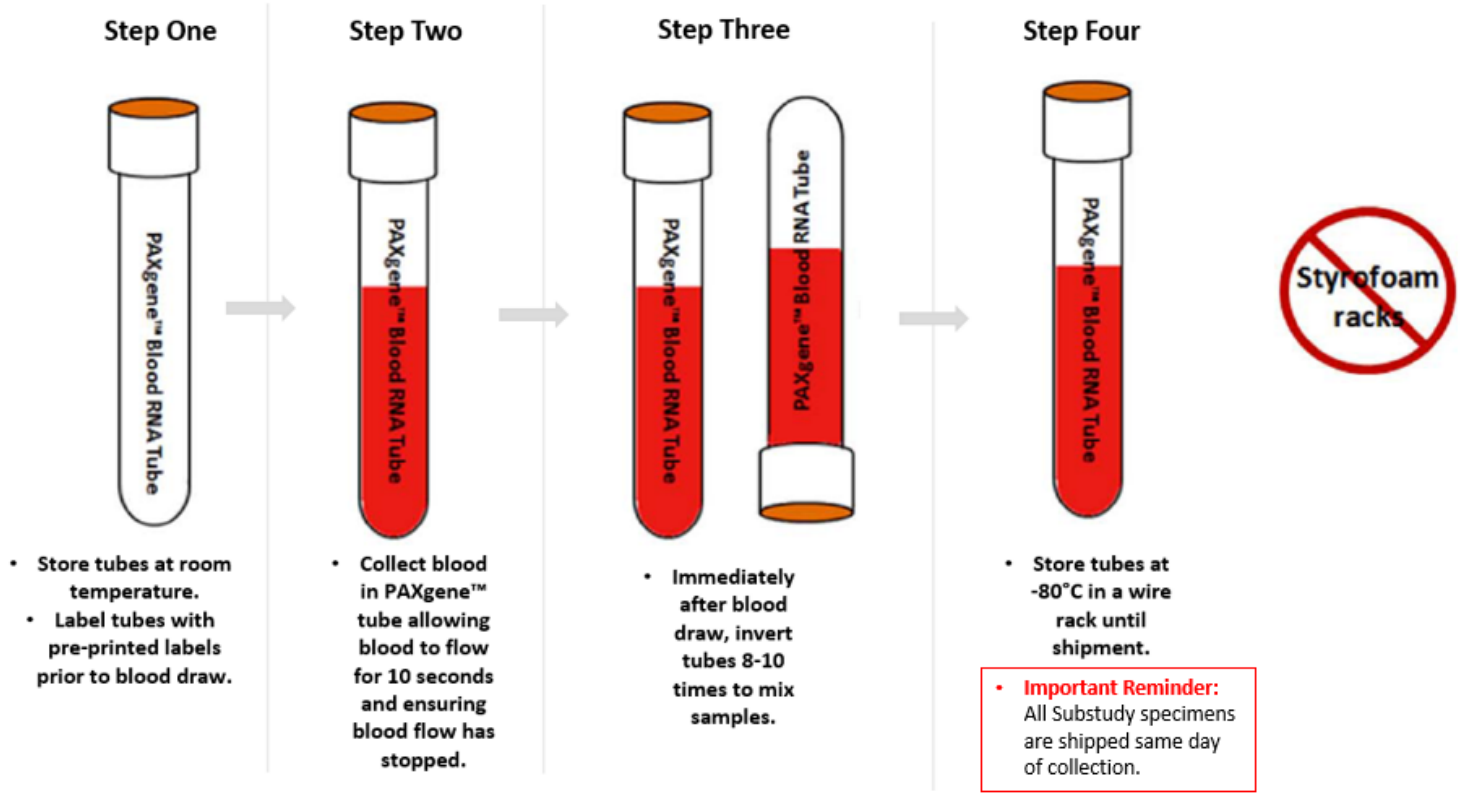
   The following techniques shall be used to prevent possible backflow:
   a. Place participant's arm in a downward position.
   b. Hold tube in a vertical position, below the participant's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube 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 on dry ice to NCRAD. Complete remainder of the Biological Sample and Shipment Notification Form (Appendix D).

RNA Preparation (2.5ml PAXgene™ Tube)



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

- Collect blood in PAXgene™ 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.

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

- **Important Reminder:** All Substudy specimens are shipped same day of collection.

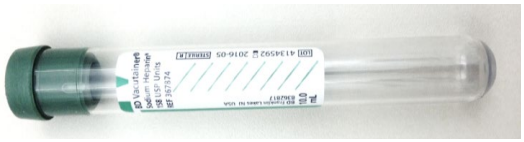
Important Note: Ensure all tubes are not expired prior to collection and processing of samples.

13.5 Sodium Heparin (Green-Top) Blood Collection Tube (10 mL) for collection of Peripheral Blood Mononuclear Cells (PBMC) x 5

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

Once drawn, Sodium Heparin tubes MUST be shipped to NCRAD <u>on the day of collection</u> via UPS Next Day Air. This is to ensure the specimen has the most viable cells available at extraction.

These samples should only be collected Monday-Thursday. Please DO NOT collect these samples on Fridays.



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

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 LIFE-DSR ID Label and pre-printed "**PBMC**" Collection Tube Label on the green-top NaHep tubes.

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

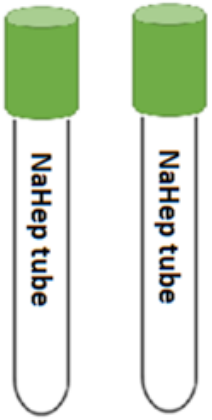
4. Immediately after blood collection, gently invert the tubes 8-10 times to mix sample.

5. Seal the Sodium Heparin tubes in the ambient shipment kit. There should be no more than 3 Sodium Heparin tubes in each ambient shipment kit. Remember to add one (1) temperature monitor per ambient shipping kit. Temperature monitor will be placed in Styrofoam cooler with tubes. Please see directions below.

6. Ship the unprocessed tubes ambient to NCRAD. **Samples must be shipped the same day as collection. Samples must be received the following day after collection. Do NOT draw or ship ambient samples on Friday. Only Monday-Thursday collection and same day shipping.**

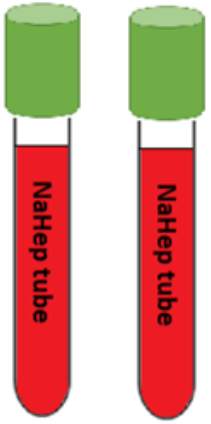
PBMC Preparation (10ml Sodium Heparin Tube x 5)

Step One



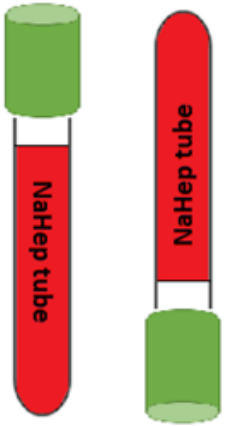
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



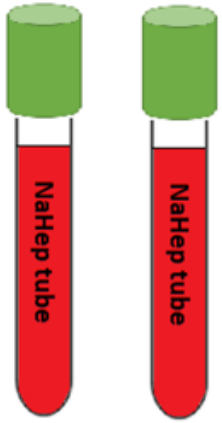
- Collect blood in Sodium Heparin Tubes allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four



- Store tubes at room temperature until shipment.
- <u>Ship ambient same day as blood draw</u>

- **Important Reminder:** All Substudy specimens are shipped same day of collection.

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

14.0 CEREBROSPINAL FLUID COLLECTION AND PROCESSING

There are general guidelines to follow in regard to CSF Collection. However, please follow and review local LP guidelines.

- Do NOT use any extension tubing due to the tendency of manufactured plastic tubing to bind beta amyloid peptides and other important AD biomarkers.
- 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 (Section 11.1), including pelleted dry ice. Check expiration dates on all supplies, especially lidocaine.

14.1 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.

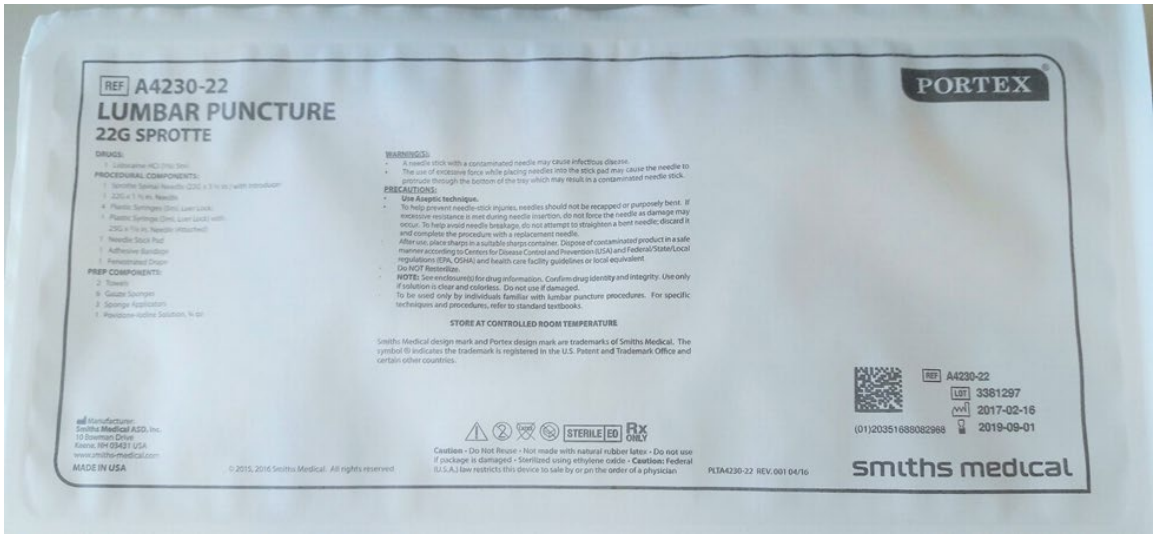
14.2 Performing the LP

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

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

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

Lumbar Puncture Tray Kit Images



Exterior of LP Tray provided by NCRAD which contains the 22-gauge Sprotte Needle with Introducer

Interior of LP Tray Provided by NCRAD

Close up of Sprotte Spinal Needle (22-gauge x 3 ½ in.) with Introducer

(24 gauge is equivalent but with lavender top needle)

TOUCH ONLY THE OUTSIDE OF THE PAPER WRAPPER

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

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

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.

Important Note

Follow local good clinical practice procedures and local SOPS for conduct of LP procedure.

Step by Step Summary of CSF Collection Procedure

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

Ensure all samples collected are appropriately labeled.

1. Print Appendix E: CSF Sample and Shipment Notification Form.
2. Confirm all supplies, including dry ice (~10 lbs.) and wet ice, are available.
3. CoolCell® should be cooled overnight in a 4 ℃ refrigerator and/or kept stored in a 4 ℃ refrigerator.
    - For optimal results, CoolCell® container should be at the same temperature as your cell suspensions.
4. Label the (13) smooth orange cap cryovials and (1) blue cap cryovial with provided CSF Cryovial Labels. Label the (1) self-standing cryogenic vial (2 mL) with provided collection tube label. Label the 15 mL conical that will store the self-standing cryogenic vial (2 mL) holding CSF pellet with a LIFE-DSR ID Label. Write Patient ID on labels before placing on tube. Do **NOT** label the (2) 15 mL conicals used for collecting CSF until step 9a.

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 20 mL CSF at the L3/L4 position (or adjacent position) using a 22- gauge Sprotte spinal needle via gravity flow with participant in upright position (or document alternate method on CSF Sample and Shipment Notification Form) following these steps:
   a. Collect initial 1-2 mL using a 15 mL conical tube. 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 20 mL into an **UNLABELED STERILE** 15 mL polypropylene tube for CSF analysis. If not bloody, transfer first 1-2 mL into yellow cap cryovial for local lab. If 20 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.
      i. **If <15 mL or less is collected, use only (1) 15 mL conical for collection.**
      ii. **If 15.1 mL or greater is collected, split the volume of CSF collected between (2) 15 mL conicals.**
   b. 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 tubes 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.
   c. 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.

9. As one person takes the immediate post procedure vital signs, a second person should process the CSF as follows:
   a. Label the 15 mL polypropylene tube(s) with LIFE-DSR ID Labels. Write Patient ID on labels before placing on tubes. Draw an arrow on the tube cap. This will help to locate the cell pellet after centrifugation.
   b. 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 (350 x g, 10 min, 4°C) to pellet cells. **If 15 mL or less is drawn, use counterweight blank tube (water) in place of empty conical tube.** Be certain to place the tubes inside the centrifuge with the arrowhead pointing to the **outside** of the centrifuge.

c. After centrifugation, remove the tubes and locate the cell pellet. DO **NOT** remove the tube cap until this is completed. The pellet will be located at the bottom of the tube along the wall facing the arrowhead. With a marker, draw an arrow pointing to the pellet for easier identification, as it may be difficult to see otherwise. Place on ice.

d. Using a clean transfer pipette, transfer CSF from 15 mL conical tube(s) into a 50 mL conical tube:

   i. Two 15 mL conical tubes collected: leave approximately 100 µL of CSF with pelleted cells at the bottom of each 15 mL centrifuged tube

   ii. Less than 15 mL collected (one tube): leave approximately 100 µL of CSF with pelleted cells at the bottom of 15 mL centrifuged tube

e. Keep 15 mL conical tube(s) with cell pellet on wet ice and gently invert the 50 mL conical tube 3-4 times to mix the sample.

f. Place sample on wet ice and process cell pellet/debris before returning to aliquot CSF supernatant into provided cryovials.

g. Cryopreservation of cell pellet/debris:

   i. Remove an aliquot of CryoStor® CS10 Cryopreservation Medium (StemCell Technologies #07930) from the refrigerator and place on wet ice. Cryopreservation media should be 4 ℃.

   ii. Place empty 2.0 mL cryogenic vial on wet ice.

   iii. Carefully resuspend the cell pellet in 1 mL of CryoStor® Medium by gently pipetting up and down near the pellet 5 times using the medium to wash the side of the pellet wall. Transfer all the cell suspension from the first 15 mL conical tube to the second 15 mL conical tube and resuspend the cell pellet the exact same way. Try not to touch the side of the conical with the pipette tip and **avoid creating bubbles/foam during this process.**

   iv. Transfer all the cell suspension to the empty 2.0 mL cryogenic vial. Recap the vial. You should have approximately 1.2-1.4 mL of CryoStor®/CSF/cells in the cryogenic vial.

   v. Place cryogenic vial in a CoolCell® well. Each well should contain a filled vial. If any empty spaces remain, fill each empty well with a Corning CoolCell® Filler Vial (2 mL vial, part number 432076).

   vi. Fully seal the lid on CoolCell® container.

vii. Place CoolCell® container upright into a -80 ℃ freezer or dry ice locker. Ensure that there is at least one inch of free space clearance around and under the CoolCell® container. Presence of snow and ice buildup in a freezer will interfere with the freezing.

viii. Leave samples in the CoolCell® for at least 2 hours before shipping on dry ice. Important: **Under no circumstance should a specimen be packaged for shipment with <2 hours in the CoolCell.**

ix. Always allow CoolCell® to warm back to room temperature (overnight is best) before cooling it again to 4 ℃.

x. See video here under "Study Resources" in the "Related Video Tutorials" tab: NCRAD - LIFE-DSR Active Study Page

Note: In the video, a serological pipette is used. NCRAD provides a plastic transfer pipette which is fine to use.

h. Return to the 50 mL conical and gently invert the 50 mL conical tube 3-4 times to mix the sample. Aliquot 1.5 mL CSF into the smooth orange-cap cryovials. If a residual aliquot is created, aliquot into blue cap cryovial. Document specimen number and volume on CSF Sample Notification Form.

i. Within 2 hours of CSF collection, samples need to be spun, aliquoted and in the freezer. Store CSF aliquots at -80°C until shipment. Record time of freezing on CSF Sample and Shipment Notification Form.

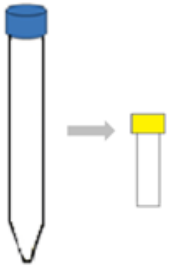
10. Provide food and drink to participant (participant may lay flat for 60 minutes to minimize the chance of a post-LP headache).

CSF Preparation (20 ml total) - Supernatant

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

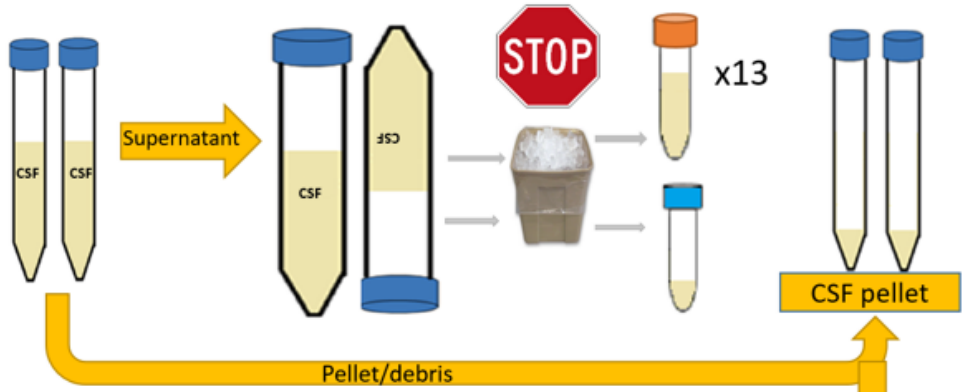
Step Four

- Place samples upright on wet ice until centrifugation begins.

Step Five

- Preferably within 15 minutes of collection, centrifuge samples at 4°C at 350 x g for 10 minutes.

Step Six



- Using a clean transfer pipette, transfer CSF from both 15 ml conical tubes into a 50 ml conical tube, leaving 100ul of CSF with pelleted cells at the bottom of each 15ml centrifuged conical tube.
- Gently invert the 50 ml conical tube 3-4 times to mix the sample. PLACE ON WET ICE.
- **STOP – TAKE CELL PELLETS AND SKIP TO STEP EIGHT. COMPLETE STEPS EIGHT THROUGH THIRTEEN BEFORE RETURNING TO STEPS SIX AND SEVEN.**
- Gently invert the 50 ml conical tube 3-4 times to mix the sample again.
- 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.

Step Seven

- 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.

- **Important Reminder:** All Substudy specimens are shipped same day of collection.

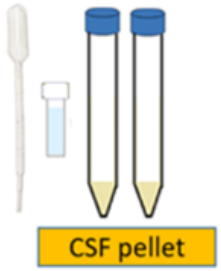
Important Note: Ensure all tubes are not expired prior to collection and processing of samples.

CSF Preparation (20 ml total) – Pellet/Cell Preparation

Step Eight

- Remove an aliquot of CryoStor® CS10 Cryopreservation Medium (StemCell Technologies #07930) from the refrigerator and place on wet ice.
- Place empty 2.0ml Cryogenic vial on wet ice.
- Ensure filler vials are at 4C. Either they can be stored in the refrigerator or they should be placed on ice for at least 20 min prior to use.

Step Nine



- Carefully resuspend the cell pellet in 1 mL of CryoStor® Medium by gently pipetting up and down near the pellet 5 times using the medium to wash the side of the pellet wall.
- Transfer all the cell suspension from the first 15 mL conical tube to the second 15 mL conical tube and resuspend the cell pellet the exact same way.
- Try not to touch the side of the conical with the pipette tip and avoid creating bubbles/foam during this process.

Step Ten

- Transfer all the cell suspension to the empty 2.0 mL cryogenic vial.
- Recap the vial.
- You should have approximately 1.2-1.4 mL of CryoStor®/CSF/cells in the cryogenic vial.

Step Eleven

- Place Cryogenic vial in a CoolCell well.
- A filler vial should be used to fill all empty spots.
- Fully seal the lid on CoolCell container.
- Place CoolCell container upright in -80°C freezer or dry ice locker.
- Ensure at least one inch of free space clearance around and under the CoolCell container.

Step Twelve

- Document specimen number and volume of Cryogenic vial on the CSF Sample Notification Form.
- Leave samples in the CoolCell for at least 2 hours at -80°C before shipping on dry ice.
- 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.

****PLEASE RETURN TO STEP SIX TO COMPLETE CSF SUPERNATANT PROCESSING**

- **Important Reminder:** All Substudy specimens are shipped same day of collection.

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

WELLNESS CHECK PHONE CALL

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

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 LEADS LP is maximized by the use of atraumatic needles. The LEADS protocol requires use of a 22-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.

Participants will be responsible for costs related to the performance of a blood patch.

15.0 PACKAGING AND SHIPPING INSTRUCTIONS

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

All Substudy samples should be collected Monday – Wednesday ONLY and shipped ON DAY OF COLLECTION!

ALL study personnel responsible for shipping should be certified in biofluid shipping (i.e., IATA certification. 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 for RNA extraction	N/A	1	Frozen / same day
Whole blood for PBMC	N/A	5	Ambient / same day
CSF Collection	1.5 mL CSF aliquots per 2.0 mL cryovial (orange cap smooth); residual volume placed in 2.0 mL cryovial with blue cap	Up to 14	Frozen / same day
	2.0 mL Cryogenic Vial with CSF Pellet/Cells (orange cap cryovial with ridges)	1	Frozen / same day

15.1 Frozen Packaging Instructions

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 SHIPPED MONDAY-WEDNESDAY ONLY ON DAY OF COLLECTION!

*****Important Note for Frozen Shipments ONLY*****

Batch shipping main study specimens and subset study specimens together: If shipping main study specimens same day as a subset collection, the 25-slot cryoboxes holding plasma and buffy coats from main study can be batch shipped with the subset specimens (PAXgene™ tube, 15 mL conical holding Cryogenic vial of CSF cells, and 48-slot cryobox holding CSF aliquots). Ensure there is ~45 lbs. of dry ice for large shippers and ~14 lbs. for small shippers.

Large Frozen Shipper – fits 5 25-slot cryoboxes and ~45 lbs. dry ice

Small Shipper – fits 3 25-slot cryoboxes and ~14 lbs. dry ice

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
  - ✓ UPS Dry Ice label, 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.

Frozen Packaging Instructions

1. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu
   Attach the following to the email:
   a. Completed Biological Sample and Shipment Notification Form and CSF Sample and Shipment Notification Form to the email notification.
   (See Appendix D and Appendix E for an example of the NCRAD sample forms)

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

2. Place all frozen labeled aliquots of CSF from the same subject in the cryovial cryobox.
   a. Each 48-slot cryobox will hold approximately 14 cryovial samples.
   b. Cryoboxes should contain all of the specimens from the same patient, per time point.

One cryobox containing CSF aliquots and residual.

3. Label the outside of the cryoboxes with the appropriate kit number label. Place 48-slot cryobox containing CSF aliquots inside biohazard bag with absorbent sheet.

4. Place Cryogenic vial containing CSF cells/pellet in labeled 15 mL conical for shipping (*cryogenic vial will not fit in the 48-slot Cryobox above due to the size of the tube*).

5. Place frozen PAXgene™ tube and labeled 15 conical containing Cryogenic vial with CSF cells/pellet inside provided bubble wrap tube sleeves, seal and place tubes inside biohazard bag with 48-slot cryobox.
6. Ensure absorbent sheet is inside the biohazard bag then seal the biohazard bag according to the instructions on the bag.

a. *The biohazard bags are large enough to contain (1) cryobox, (1) frozen PAXgene™ tube and (1) 15 mL conical holding Cryogenic vial of CSF cells/pellet from one subject's visit.*

7. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam shipping container.
8. Place the biohazard bag into the provided Styrofoam-lined shipping container on top of the dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container (as pictured below).
9. Fully cover the cryoboxes and tubes with approximately 2 inches of dry ice.
10. The inner Styrofoam shipping container must contain approximately 10 lbs. (or 4.5kg) of dry ice. The dry ice should entirely fill the inner box to ensure the frozen state of the specimens.

Full Shipping Container with Batched Samples and Dry Ice

11. Replace the lid on the Styrofoam carton. Place the completed Biological Sample and Shipment Notification Form and CSF Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape.
12. Complete the UPS Dry Ice Label
    a. Net weight of dry ice in kg (must match amount on the airbill)
    b. Do not cover any part of this label with other stickers, including pre-printed address labels.
13. Apply all provided warning labels and the pre-printed UPS return label to the outside of package, taking care not to overlap labels.

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

14. Hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off.
15. Specimens should be sent to the below address via **UPS Next Day Air ON DAY OF COLLECTION**. Frozen shipments should be sent **Monday through Wednesday** to avoid shipping delays on Thursday or Friday. UPS does not replenish dry ice if shipments are delayed or held over during the weekend.

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

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

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

Remember to complete the Biological Sample and Shipment Notification Forms (Appendix D and Appendix E (and Appendix B if batch shipping), include a copy in your shipment AND notify the NCRAD Study Coordinator by email at alzstudy@iu.edu (include UPS tracking number in email) IN ADVANCE to confirm the shipment.

15.2 Ambient Packaging Instructions

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

For ambient Sodium Heparin (Green-Top) Blood Collection Tube (5x10 ml) shipments, include no more than three tubes per shipping container and include only tubes from one participant. The ambient PBMC samples MUST be shipped the day of blood draw.

The labeled, unprocessed, sodium heparin PBMC tubes will be shipped to NCRAD as outlined below.

IMPORTANT!
AMBIENT SAMPLES MUST BE SHIPPED **MONDAY-WEDNESDAY ONLY ON DAY OF COLLECTION** **Do NOT draw blood for ambient shipments on Thursdays or Fridays!**

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

- The primary receptacle (sodium heparin tube) must be leak proof and must not contain more than 10 ml total.
- The secondary packaging (foam box) must be leak proof.
- Absorbent material must be placed between the primary receptacle (sodium heparin tube) and the secondary packaging (foam box). 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

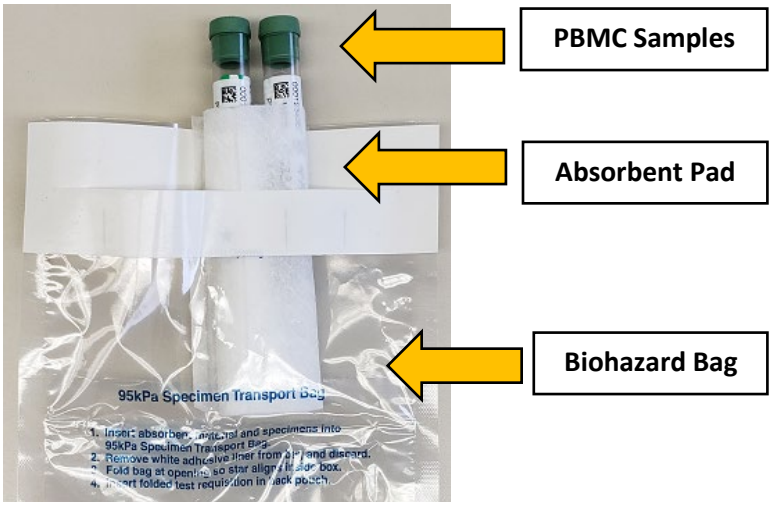
Ambient Sodium Heparin (Green-Top) Blood Collection Tube (10 mL) shipments should be considered as Category B UN3373 and as such must be tripled packaged and compliant with the IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

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.

NCRAD Packaging Instructions (Ambient Shipments)

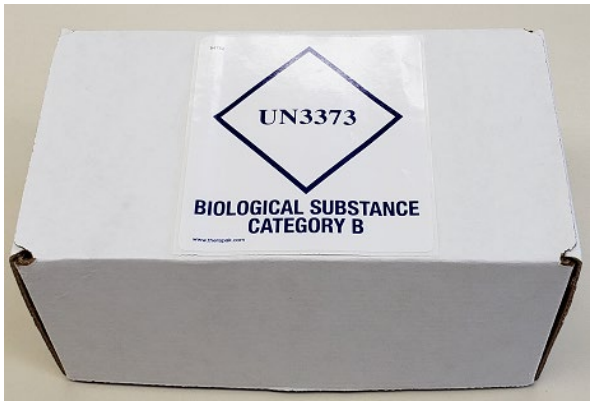
1. **Place refrigerant pack in the refrigerator, ~4°C, 24 hours prior to shipment.**
2. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu
    a. Complete and attach the Biological Sample and Shipment Notification – Substudy Form to the email. (See Appendix D for an example of the form)
    b. If email is unavailable please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.
3. Place filled and labeled sodium heparin (green-top) tubes within the slots in the absorbent pad provided, and place into the plastic biohazard bag with absorbent sheet. Only place up to 3 tubes in each ambient shipper.



4. Remove as much air as possible from the plastic biohazard bag and seal the bag according to the directions printed on the bag.
5. Place Kit Number Label on biohazard bag.
6. Place the refrigerant pack into the cooler on top of the filled biohazard bag.

7. **Place one (1) temperature monitor in Styrofoam cooler with tubes (See Page 45)**

8. Place the lid onto the cooler.
9. Place the cooler in the provided small IATA Shipping Box.
10. Place an extra copy of the emailed "Biological Sample and Shipment Notification Form" within the shipping box along with a list of contents form.
11. Close shipping box. Label the outside of the cardboard box with the enclosed UN3373 (Biological Substance Category B) label.

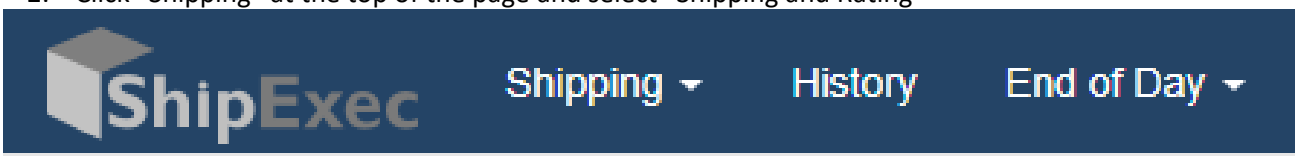


12. Place the closed, labeled shipping box within a UPS Laboratory Pak. **Seal the UPS Laboratory Pak.**
13. Use UPS tracking to ensure the delivery occurs as scheduled and is received by NCRAD.

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

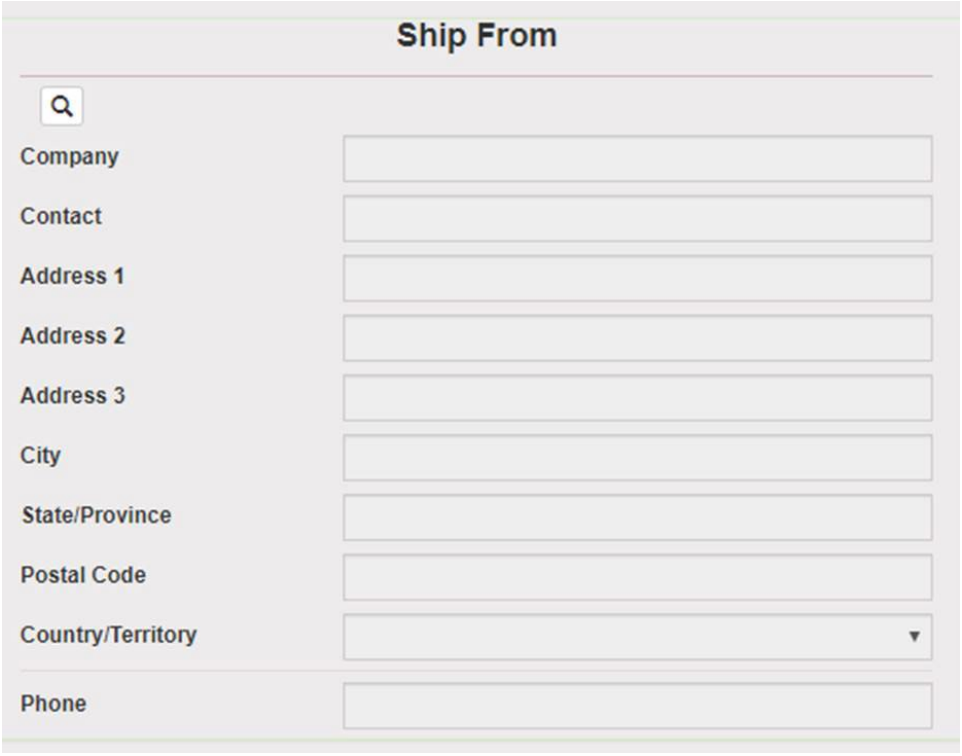
15.3 Frozen and Ambient Shipping Instructions

1. Log into the ShipExec Thin Client at kits.iu.edu/UPS.
   a. If a new user or contact needs access, please reach out to your study contact for access.
2. Click "Shipping" at the top of the page and select "Shipping and Rating"



3. Select your study from the "Study Group" drop down on the right side of the main screen. Choosing your study will automatically filter the address book to only addresses within your study.

4. Click on the magnifying glass icon in the “Ship From” section to search for your shipping address.



   a. Search by Company (site), Contact (name), or Address 1 (first line of your site’s street address). Click Search.
   b. Click Select to the left of the correct contact information.
5. Verify that both the shipping information AND study reference are correct for this shipment.
   a. If wrong study contact or study reference, click Reset in the bottom right of the screen to research for the correct information.
6. Enter Package Information
   a. Ambient shipments
      i. Enter the total weight of your package in the “Weight” field and leave the “Dry Ice Weight” field empty.
   b. Frozen shipments
      i. Enter the total weight of your package in the “Weight” field.
      ii. Enter the dry ice weight in the “Dry Ice Weight” field.
      iii. If the “Dry Ice Weight” field is higher than the “Weight” field, you will receive an error message after clicking “Ship” and need to reenter these values.
   c. Click Ship in the bottom right of the page when complete.
7. If your site does not already have a daily UPS pickup, you will need to schedule one
   a. “Click the blue Pickup Request button. Enter the earliest pickup time and latest pickup time in 24-hr format.
   b. Give a name & phone number of someone who the UPS driver can call if having issues finding the package

   c. Give the Floor and Room Number (if needed) to be as descriptive as possible where this package needs to be picked up from. Click Save

8. Print the airbill that is automatically downloaded.
   a. To reprint airbill, click History at the top left of the page.
   b. Click Detailed Report from the dropdown menu on the right side of the page.
   c. Enter tracking number if known. Otherwise, search by ship date. Click Search.
   d. Click print icon on right side of the tracking number line.
9. Fold airbill, and place inside plastic UPS sleeve.
10. Peel the back off of the UPS sleeve and stick the sleeve to the package.
11. A UPS Pickup is automatically scheduled at the address you are shipping from, and the pickup is charged to NCRAD.
    a. If shipment occurs too late in the day for an automatic UPS pickup, you will receive an email stating that the pickup could not be scheduled, and you will need to make other arrangements.

16.0 APPENDICES LIST

Appendix C
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

alzstudy@iu.edu

Appendix D

Participant ID: DSR __ __ __ __ __ __ __ __

Biological Sample and Shipment Notification Form - SubStudy

Please email or fax this from prior to the date of shipment.

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

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

From: _____ Date: _____

Phone: _____ Email: _____

Study: LIFE-DSR **Kit #:** KIT BARCODE

Visit (circle one): **BASELINE** **MONTH 16** **MONTH 32**

Sex: ☐ M ☐ F **Year of Birth:** _____

Blood Collection:

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

Blood Processing:

RNA (PAXgene Tube)

Total volume of blood drawn into a 1 x 2.5 mL PAXgene RNA tube: _____ mL

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

PBMC (NaHep Green Top Tube)

Original volume drawn (5x10mL PBMC tube): _____ mL

Notes:

Version (04.2021)

Appendix E

Participant ID: DSR _ _ _ _ _ _ _ _

CSF Sample and Shipment Notification Form - SubStudy

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: _____ Date: _____

Phone: _____ Email: _____

Study: LIFE-DSR

KIT BARCODE

Visit (circle one) : **BASELINE** **MONTH 16** **MONTH 32**

Sex: ☐ M ☐ F **Year of Birth:** _____

CSF Collected? ☐ Yes ☐ No

UPS tracking #: _____ **Gauge needle used for LP:** ☐ 22G ☐ 24G

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: ☐ Gravity Method **OR** ☐ Aspiration

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 1.5 mL aliquots (supernatant) created (up to 14 total): **(Orange cap cryovials):**	_____ x 1.5 mL
If applicable, volume of CSF residual aliquot (less than 1.5 mL): **(Blue cap cryovial):**	_____ mL
If applicable, specimen number of residual aliquot tube: **(Last four digits):**	_____
Volume of CSF pellet in Cryogenic vial (less than 2 mL): **(Orange cap w/ ridges):**	_____ mL
Specimen number of Cryogenic vial (CSF pellet) **(Last four digits):**	_____
Time frozen - Supernatant: _____ [HHMM]	Time frozen - CSF Pellet: _____ [HHMM]
Storage temperature of freezer:	_____ °C

Notes: _____

Ver: 05.2021

Approval & Signatures
Document Name: Biofluid Collection, Processing and Shipment Manual of Procedures *Version: 6.2* *Version Date: 3/9/2023*
Sponsor Printed Name: Aisha Vanderhorst Signature: Aisha Vanderhorst Date: 8MAR2023
Technical Lead Printed Name: Michael C. Edler Jr. Signature: [signature] Date: 03/09/2023
Author/Responsible Lead Printed Name: Zoë Potter Role: Clinical Research Coordinator Signature: Zoë D. Potter Date: 03/9/2023