ALLFTD Manual of Procedures Update: Version 04.2025

Section	Change
Document Footer	The version date was updated for this amendment.
Throughout Document	Minor changes made to phrasing and wording. No alterations to procedures were made.
6.1	New label examples updated from last revision, now including ‘COLLECT’ and ‘ALIQUOT’

ARTFL LEFFTDS Longitudinal Frontotemporal Lobar Degeneration (ALLFTD)

in collaboration with the

National Centralized Repository for Alzheimer's Disease and Related Dementias

Biospecimen Collection, Processing, and Shipment Manual

Version (04.2025)

Table of Contents

1.0 Abbreviations

ALLFTD	ARTFL LEFFTDS Longitudinal Frontotemporal Lobar Degeneration
CITI	Collaborative Institutional Training Initiative
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
LP	Lumbar Puncture
NaHep	Sodium Heparin
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PBMC	Peripheral Blood Mononuclear Cell
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RNA	Ribonucleic Acid
RPM	Revolutions Per Minute

2.0 Purpose

The purpose of this manual is to provide ALLFTD staff (PIs, study coordinators, and the sample collection and processing teams) at the various study sites with instructions for collection and submission of biological samples for ALLFTD study visits. It includes instructions for biospecimen submission to the National Centralized Repository for Alzheimer's disease and Related Dementias (NCRAD) located at Indiana University. The following samples may be collected at each study visit:

- Plasma
- Buffy Coat (for DNA extraction)
- PBMC
- Serum
- RNA
- CSF (optional)

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

These procedures are relevant to all study personnel responsible for processing blood specimens to be submitted to NCRAD for the ALLFTD protocol.

3.0 NCRAD Information

3.1 NCRAD Contacts

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

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

Corinne Kim, Study Coordinator
Phone: 317-274-7417
Email: kimcor@iu.edu

Jazmyn Dickinson, Study Coordinator
Email: jazdicki@iu.edu

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

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

3.2 NCRAD Hours of Operation

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

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

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

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

3.3 NCRAD Holiday Observations

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

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

4.0 ALLFTD 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

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 ≥ 1500 rcf (1500 x g) with refrigeration to 4°C
- -80°C Freezer

In order to batch-ship frozen specimens, you must provide:

- Pelleted dry ice (approximately 45 lbs per shipment)

4.2 Biospecimens Sent to NCRAD

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

Ambient samples are to be submitted according to the shipping methods outlined in Section 8.2. Guidelines for the processing, storage location, and timing of sample collection are listed by site in the tables below.

Biospecimens collected include whole blood and CSF. Please refer to the below table for the biospecimen schedule for both the Longitudinal and Biofluid-Focused Arm.

	RAVE Cycle (ALL)
DNA (Buffy Coat)	X
Plasma	X
PBMC	X
Serum	X
RNA	X
CSF*	X

*Optional for all participants

Whole blood will be collected into eight different collection tubes (lavender-top EDTA tubes, green-top sodium heparin tubes, red-top serum determination tube, and PAXgene™ tubes).

The lavender-top EDTA tubes are processed locally into plasma and buffy coat fractions, aliquoted, frozen at the study site, and then shipped to NCRAD.

The green-top Sodium Heparin tubes (for PBMCs) are kept ambient without further processing and shipped the same day of the blood draw.

The red-top serum determination tube is processed locally into serum fractions, aliquoted, frozen at the study site, and then shipped to NCRAD.

The PAXgene™ tubes are frozen locally without further processing and then shipped to NCRAD.

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

Frozen samples (plasma, buffy coat, serum, RNA, and CSF) 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 Informed Consent

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

4.4 Biospecimen Collection Charts

4.4.1 Blood Collection

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the **Biological Sample and Shipment Notification Form (see Appendix B).** Submit a copy to NCRAD with a reason provided for the omission. See Appendix H if the sample collection below cannot be followed for a Biofluid-Focused Arm Visit.

Sample Type	Tube Type	Study Visits Collecting Biospecimens	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Typical # of tubes sent to NCRAD	Ship
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	**All Cycles**	**3**	**3**	**N/A**	**N/A**
	PLASMA: 2 ml cryovials with lavender caps (residual volume placed in 2 ml cryovial with blue cap)	All Cycles	10 (9 Lavender Cap, 1 Blue Cap Cryovial)	1.5 ml plasma aliquots per 2.0 ml cryovial	8-10	Frozen
	BUFFY COAT: 2 ml cryovial with a clear cap	All Cycles	3	1 ml buffy coat aliquot per 2.0 ml cryovial	3	Frozen
Whole blood for PBMC isolation	**Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)**	**All Cycles**	**2**	**N/A**	**2**	**Ambient**
Whole blood for isolation of serum	**Serum Determination (Red-Top) Blood Collection Tube (10 ml)**	**All Cycles**	**1**	**1**	**N/A**	**N/A**
	Serum: 2 ml cryovials with red caps (residual volume placed in 2.0 ml cryovial with blue cap)	All Cycles	4 (3 Red Cap, 1 Blue Cap Cryovial)	1.5 ml Serum Aliquots Per 2.0 ml cryovial	3-4	Frozen
Whole blood for RNA isolation	**PAXgene™ Blood Collection Tube (2.5 ml)**	**All Cycles**	**2**	**N/A**	**2**	**Frozen**

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

4.4.2 Cerebrospinal Fluid

If a CSF sample *is or is not* obtained at a particular visit, this should be recorded in the CSF section of the **Biological Sample and Shipment Notification Form (see Appendix B).** See Appendix H if the sample collection below cannot be followed for a Biofluid-Focused Arm Visit.

Sample Type	Tube Type	Study Visit Collecting Biospecimens	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Typical # of tubes to NCRAD	Ship
CSF	**50 ml screw top centrifuge tubes with blue caps**	**All participants in the longitudinal and biofluid arm**	**2**	**N/A**	**N/A**	**N/A**
	2 ml cryovial with orange caps (residual volume placed in 2 ml cryovials with blue caps)	**All participants in the longitudinal and biofluid arm**	16 (15 Orange Cap, 1 Blue Cap Cryovial)	1.5 ml CSF aliquots per 2 ml cryovials	13-17	Frozen
	2 ml cryovial with orange caps	**All participants in the longitudinal and biofluid arm**	1	1.5 ml CSF aliquots per 2 ml cryovials	0	To local lab

5.0 Specimen Collection Kits, Shipping Kits and Supplies

There are 6 ALLFTD research specimen collection kits, they are:

- Blood Kit (for both Longitudinal and Biofluid-Focused Arm Visits)
- CSF Kit (for both Longitudinal and Biofluid-Focused Arm Visits)
- Shipping Kit for Frozen Samples
- Take Home/Redraw Kit for Green Top- Sodium Heparin Tube
- Take Home/Redraw Kit for Lavender Top- EDTA Tube
- ALLFTD Supplemental Supplies Kit

Research specimen collection kits as well as clinical lab supplies (except pelleted dry ice and equipment supplies listed above in Section 4.1) will be provided by NCRAD. These materials include blood tubes, Lumbar Puncture trays (when applicable), boxes for plasma/buffy coat/CSF aliquots storage and shipment, as well as partially completed shipping labels to send materials to NCRAD. Barcoded kit labels, site and RAVE ID labels, collection tube labels, and aliquot tube labels will all be provided by NCRAD. Collection tube labels and aliquot tube labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 6.1.

5.1 NCRAD 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.*

ALLFTD Blood Kit (for both the Longitudinal and Biofluid-Focused Arms)

Quantity	ALLFTD Blood Kit Components
3	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
2	Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)
1	Serum Determination (Red-Top) Blood Collection Tube (10 ml)
2	PAXgene™ Blood Collection Tube (2.5 ml)
1	Screw-top conical tube with blue cap (50 ml)
9	Cryovial tube (2 ml) with lavender cap
3	Cryovial tube (2 ml) with red cap
3	Cryovial tube (2 ml) with clear cap
2	Cryovial tube (2 ml) with blue cap
4	Disposable graduated transfer pipette
25	Pre-printed labels for blood collection and aliquot tubes
5*	Pre-printed labels with kit number
9**	Labels for handwritten Site and RAVE ID
1	Cryovial tube box (holds up to 25 cryovials)
1	Shipping Supplies for ambient shipment of PBMC: Plastic biohazard bag with absorbent sheet Small IATA shipping box with insulated cooler Small refrigerant pack Aqui-Pak 2 tube absorbent pouch UN3373 Biological Substance Category B label List of contents card

	UPS Clinic Pak

***2 labels for ambient / frozen shipment forms, 2 labels for ambient / frozen biohazard bags, 1 label for cryobox**

****8 RAVE ID labels used for blood collection tube, 1 extra**

ALLFTD CSF Kit (optional)

Quantity	ALLFTD CSF Kit Components
16	Cryovial tube (2 ml) with orange cap
1	Cryovial tube (2 ml) with blue cap
1	Lumbar Puncture tray with 24G Sprotte needle
2	Screw-top conical tube with blue cap (50 ml)
4	Disposable graduated transfer pipette
1	Cryovial tube box (holds up to 25 cryovials)
17	Pre-printed labels for CSF collection and aliquot tubes
4	Pre-printed labels with kit number

Frozen Shipping Supply Kit

Quantity	Frozen Shipping Kit Components
8	Small plastic Biohazard bag with absorbent sheets
1	Shipping box/Styrofoam container
1	Warning label packet with dry ice sticker
8	Bubble wrap sleeves (2 connected slots each)

Green Top-Sodium Heparin Tube Redraw/Take Home Kit (optional/as needed)

Quantity	Ambient Shipping Supply Components
2	Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)
2	Pre-printed label for blood collection tube
2	Label for handwritten Site and RAVE ID
1	Shipping Supplies for ambient shipment of PBMCs: Plastic biohazard bag with absorbent sheet Small IATA shipping box with insulated cooler Small refrigerant pack Aqui-Pak 2 tube absorbent pouch UN3373 Biological Substance Category B label List of contents card UPS Clinic Pak

Lavender Top-EDTA Tube Redraw/Take Home Kit (optional/as needed)

Quantity	Ambient Shipping Supply Components
1	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
1	Pre-printed label for blood collection tube
1	Label for handwritten Site and RAVE ID
2*	Pre-printed labels with kit number
1	Shipping Supplies for ambient shipment of EDTA: Plastic biohazard bag with absorbent sheet

	Small IATA shipping box with insulated cooler Small refrigerant pack Aqui-Pak 1 tube absorbent pouch UN3373 Biological Substance Category B label List of contents card UPS Clinic Pak

*** 1 label for ambient shipment form, 1 label for biohazard bag**

ALLFTD Supplemental Supply Kit

Quantity	ALLFTD Supplemental Kit Components
5	Cryovial tube box (holds up to 25 cryovials)
10	Bubble wrap sleeve
5	Small plastic Biohazard bag with absorbent sheet
25	Cryovial tube (2 ml) with red cap
10	Cryovial tube (2 ml) with blue cap
25	Cryovial tube (2 ml) with orange cap
50	Cryovial tube (2 ml) with lavender cap
25	Cryovial tube (2 ml) with clear cap
1	Needle – Sprotte Needle w/ Introducer 24G x 90mm, 1mm x 30mm
10	Screw-top conical tube with blue cap (50 ml)
10	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
5	Serum Determination (Red-Top) Blood Collection Tube (10ml)
8	PAXgene™ Blood Collection Tube (2.5 ml)
8	Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)
5	Disposable graduated transfer pipette
5	Warning label packet

We realize there may be instances where additional supplies are needed; therefore, one supplemental kit will be provided with the initial kit shipment. Replacement supplemental kits can be requested on the kit web site. In addition, individual supplies can be requested. The following table lists all individual supplies that can be requested and the quantities at which they are available.

Individual Supplies*

Quantities	Item
5 or 10	Microcentrifuge tube box (holds up to 25 cryovials)
10, 25	Cryovial tube (2 ml) with blue cap
10, 25	Cryovial tube (2 ml) with orange cap
25, 50	Cryovial tube (2 ml) with lavender cap
25, 50	Cryovial tube (2 ml) with clear cap
25, 50	Cryovial tube (2 ml) with red cap
5, 10	Bubble wrap sleeve (2 connected slots each)
5, 10	Small IATA shipping box for ambient shipping
1, 2, 3	Shipping container for dry ice shipment (shipping and Styrofoam box)
1, 5	Needle – Sprotte Needle w/ Introducer 24G x 90mm, 1mm x 30mm

5, 10	Individually Packaged Sterile 50ml Conical Tube
5, 10	Plastic biohazard bag
5, 10, 15	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
5, 10, 15	Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)
5, 10, 15	Serum Determination (Red-Top) Blood Collection Tube (10ml)
5, 10, 15	PAXgene™ Blood Collection Tube (2.5 ml)
30, 60	Disposable graduated transfer pipette
5, 10	Warning label packet
By Request	RAVE ID label

*Available upon request on the kit web-site

5.2 Kit Supply at Study Sites

Each individual site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out so you are prepared for study visits; it can take up to 3 weeks for requested kits/supplies to be delivered to you. Please go to: http://kits.iu.edu/allftd to request additional kits and follow the prompts to request the desired supplies.

Options include ordering specific number of kits (ALLFTD Blood Kit, Optional NCRAD CSF, Frozen Shipping Supply Kit and/or an ALLFTD Supplemental Kit) or individual supplies. All kits can be used for any ALLFTD participant in either the Longitudinal or Biofluid-Focused Arms.

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

6.0 Blood Collection and Processing Procedures

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

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

1. **EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Buffy Coat and Plasma (3)**
2. **Sodium Heparin (Green-Top) Blood Collection Tube (10 ml) for PBMC (2)**
3. **Serum Determination Tube (Red Top) for Serum**
4. **PAXgene™ Blood Collection Tubes for RNA (2)**

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

6.1 Labeling Samples for NCRAD

Label Type Summary

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

Kit Number Labels link all specimens collected from a single participant at one visit. They should be placed in the designated location on the Sample and Shipment Notification Form (either Blood or CSF). There will be a different Kit Number for the Blood Kit and the CSF Kit. The Kit Number Label will also need to be placed on the corresponding patient's cryovial box and biohazard bag, which can be used for storage and is required for sending as a frozen BATCH shipment to NCRAD.

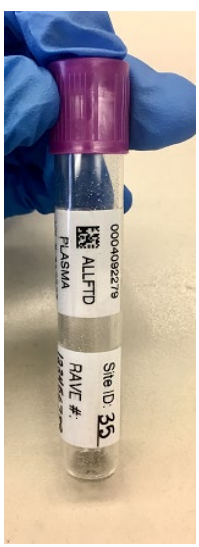
Site and RAVE ID Labels are used to document the individual's unique RAVE ID. They are placed on each of the collection tubes (EDTA, Sodium Heparin, Serum, and PAXgene™). ALLFTD staff will write-in the Site ID and participant's RAVE ID.

Place the **COLLECT label** on the collection tube. Each collection tube will contain two labels: the **Site and RAVE ID Label** and the **Collection Tube Label**. (Pictured below).

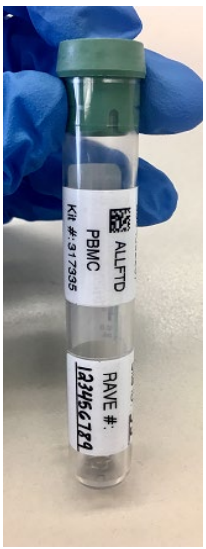
Note: The Collection Tube Labels intended for the CSF tubes will contain a kit number differing from the patient's other cycle specimens.

Place the **ALIQUOT labels** on the aliquot tube.

Note: The Aliquot Tube Labels intended for the CSF tubes will contain a kit number differing from the patient's other cycle specimens.

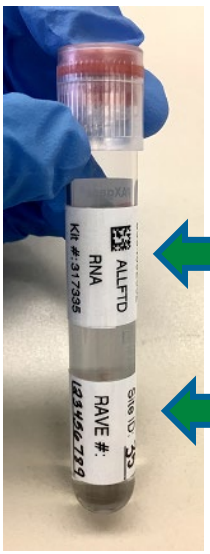


EDTA Tube



Sodium Heparin Tube

Serum Determination Tube



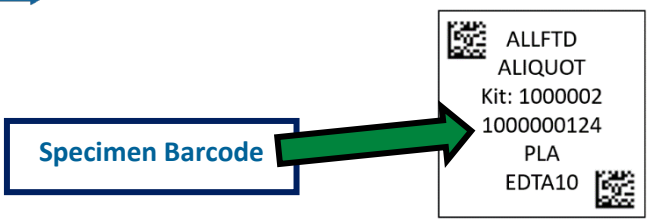
PAXgene™ Tube

Collection Tube Label

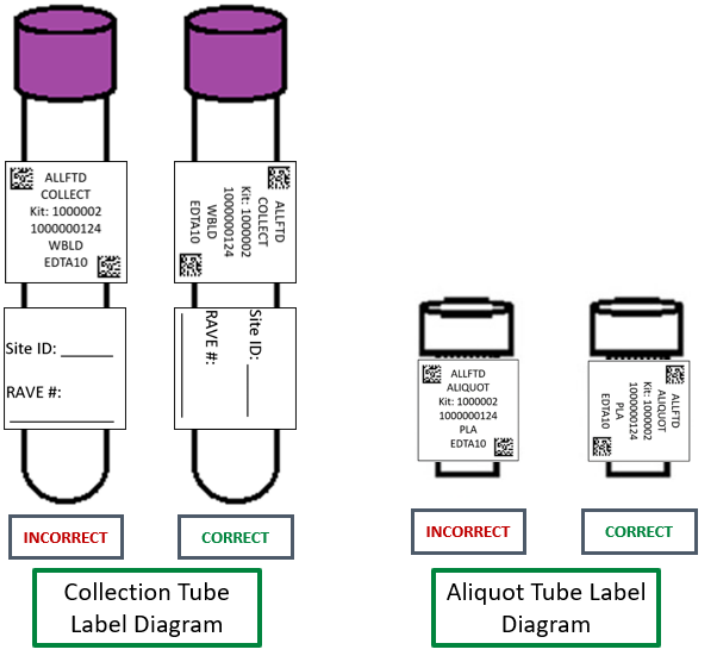
Site and RAVE ID Label

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

- Place Specimen 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 cryovial box in numerical order based on the specimen number, located at the top of the label. This ensures that no aliquot is misplaced or lost during the shipment process (see depiction below).



- Using a fine point permanent marker, fill-in and then place the Site and RAVE ID labels on the collection tubes only (EDTA, Sodium Heparin, Serum, and PAXgene™). For best adhesion results place the label on the tubes **BEFORE** sample collection, processing, or freezing. **DO NOT** place Site and RAVE ID labels on any NCRAD cryovials.
- There will be 2 types of labels on all collection tubes: Site and RAVE ID Labels and Collection and Aliquot Tube Label
- The Collection and Aliquot Tube Labels contain a 2D barcode on the left hand side of the label. Place this barcode toward the tube cap.
- Always place the label **horizontally** on the aliquot tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see attached labeling diagram).



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

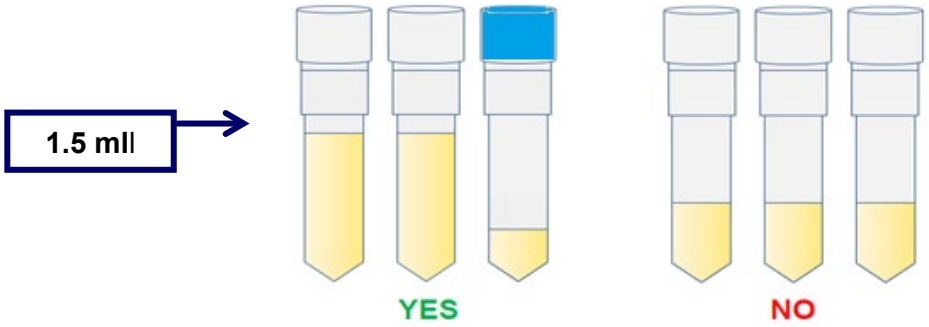
6.2 Video List

- NCRAD offers training videos that are available to assist you with the specimen processing, aliquoting, and shipping processes. The videos are available at https://ncrad.org/resource_allftd.html.
  - Frozen Shipping
  - Ambient Shipping
  - Plasma and Buffy Coat Processing and Aliquoting
  - Serum Processing and Aliquoting
  - CSF Processing and Aliquoting
  - ALLFTD MOP Training

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

In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to the assigned volume after processing is completed (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of sample.

Aliquot the remaining biologic material into a blue-cap residual tube and ship to NCRAD. Ship *all* material to NCRAD. Fill as many aliquot tubes as possible. For example, if 2.7 ml of a plasma sample is obtained, fill 1 cryovial tube with 1.5 ml, and one additional cryovial tube with the remaining 1.2 ml.



Please note: It is critical for the integrity of future studies using these 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 last four digits of the residual aliquot on the Biological Sample and Notification Form.
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.

To assist in the preparation and aliquoting of samples, colored caps are used for

the aliquot tubes. The chart below summarizes the association between cap color and type of aliquot.

Cap Color	Sample Type
Lavender	Plasma
Clear	Buffy Coat
Red	Serum
Orange	CSF Aliquot
Blue	Residual Aliquot (Plasma, Serum, or CSF)

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: three EDTA (Lavender-Top) Blood Collection Tubes (10 ml) (for processing of plasma aliquots and buffy coat aliquots). Three lavender-top tubes are collected at every study visit (both Longitudinal and Biofluid-Focused Arms) obtaining biospecimens.

1. Store empty EDTA tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use.

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

3. Place completed Site and RAVE ID and pre-printed "**PLASMA**" *collection tube label* on the three EDTA (Lavender-Top) Blood Collection Tubes (10 ml). Place pre-printed "**PLASMA**" *aliquot labels* on the (10) 2 ml cryovial tubes with lavender caps. Place pre-printed "**BUFFY COAT**" aliquot label on the (3) 2 ml cryovials with a clear cap.

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

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

The following techniques shall be used to prevent possible backflow:

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

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

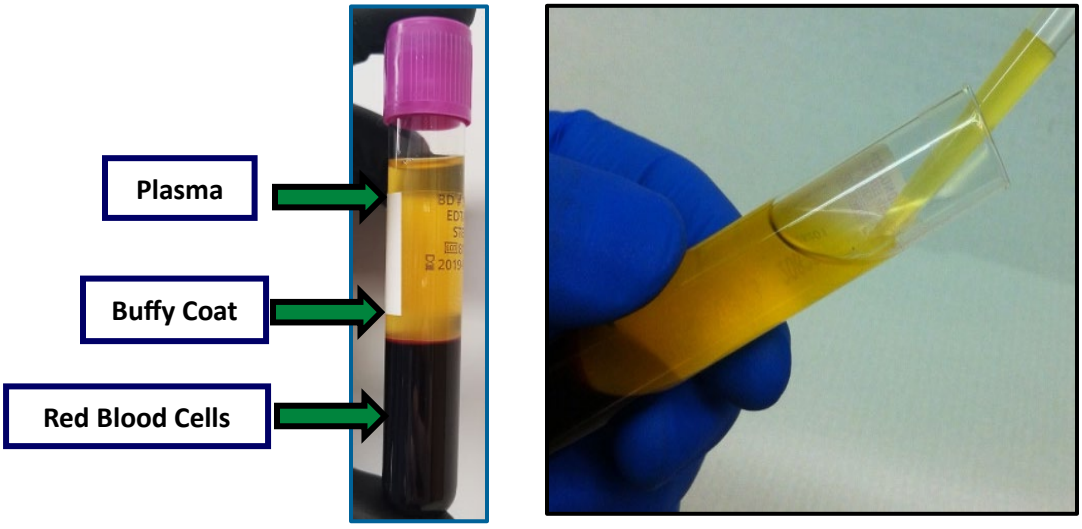
7. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8-10 times.

8. Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.

9. Centrifuge balanced tubes for 15 minutes at 1500 RCF (x g) at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM in your particular rotor).**
    a. Equivalent rpm for spin at 1500 x g
    b. While centrifuging, remember to record all times, temperatures and spin rates on the Blood Sample and Shipment Notification Form.
    c. Record time aliquoted on the Blood Sample Shipment and Notification Form.
    d. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.

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

11. Each EDTA tube should yield, on average, 4-5 ml of plasma. Using a disposable graduated transfer pipette, transfer plasma from all three EDTA tubes into a 50 ml conical tube and gently invert 3 times. Aliquot 1.5 ml plasma per cryovial. Be sure to only place **plasma** in cryovials with purple caps and labeled with **PLASMA** labels**.** Place residual plasma (<1.5 ml) in the blue-capped cryovial. **If a residual aliquot (<1.5 ml) is created, document the specimen number and volume on the Biological Sample and Shipment Notification Form.**

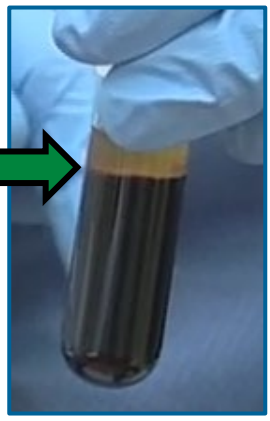


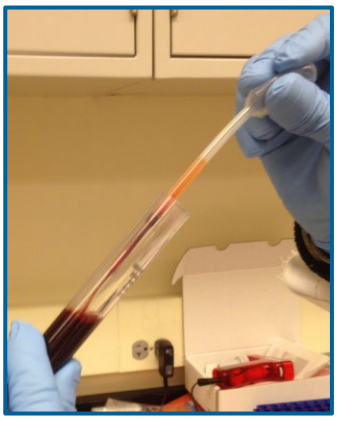
NOTE: When pipetting plasma from the plasma tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

12. Place the labeled cryovials in the 25-cell cryobox and place on dry ice. **Transfer to -80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample Shipment and Notification Form.

13. After plasma has been removed from the EDTA (Purple-Top) Blood Collection Tubes (10 ml), aliquot the buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs - see figure) from one EDTA tube into a labeled, clear-capped cryovial using a clean disposable transfer pipette. The buffy coat from each EDTA tube will be placed in a separate 2.0 ml cryovial with clear cap, so a total of three buffy coat aliquots will be submitted to NCRAD per participant, per visit. The buffy coat aliquot is expected to have a reddish color from the included RBCs. Be sure to place the buffy coat into the cryovial with the clear cap and "BUFFY COAT" label. Please place the buffy coat from only one blood tube in each cryovial.

Buffy Coat layer (mixed with RBCs)



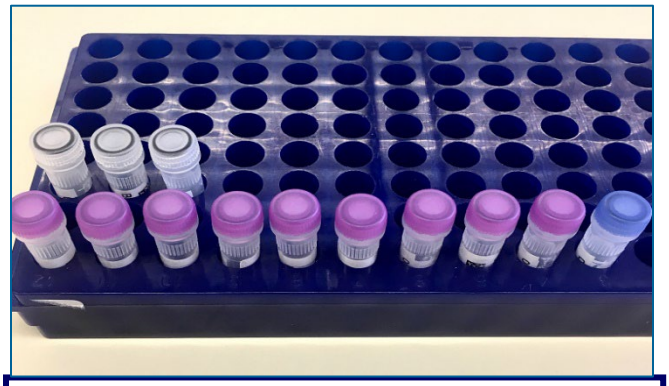


Buffy Coat aliquot (clear-capped cryovial)

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

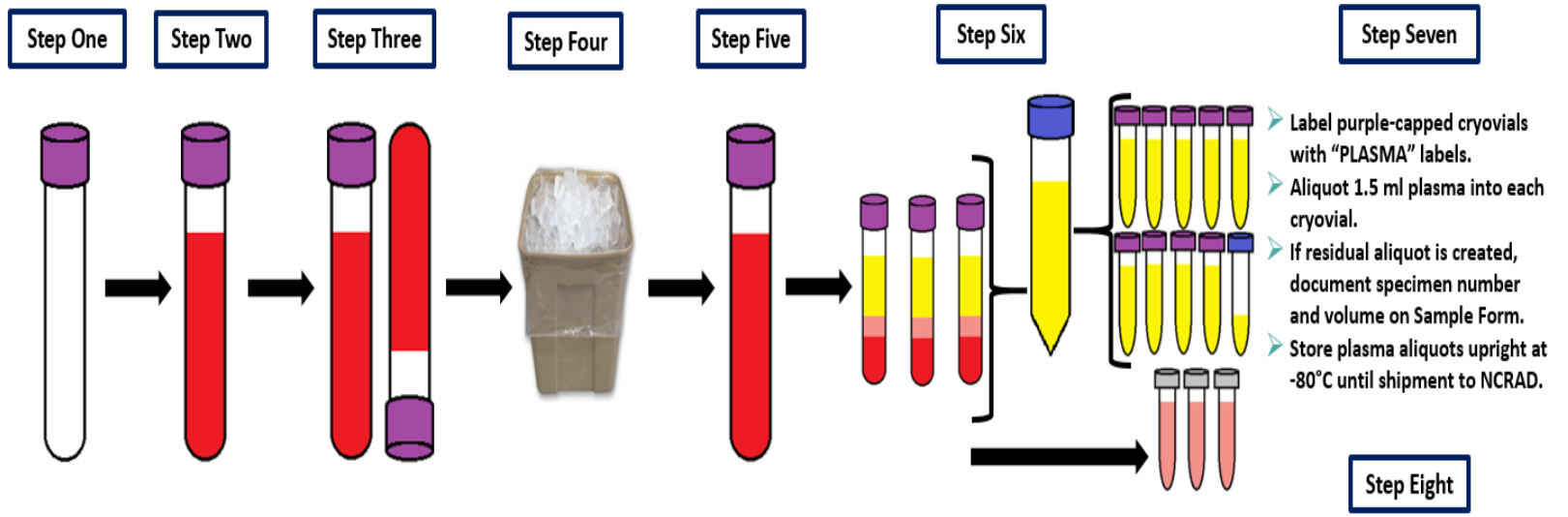
15. Record the specimen number and volumes of the EDTA tubes and corresponding buffy coat samples on the Biological Sample Shipment and Notification Form.

16. Place the labeled cryovials in the 25 cell cryobox and place on pelleted dry ice. **Transfer to -80°C Freezer when possible.** Store all samples at -80°C until shipped to NCRAD on pelleted dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.



Plasma Aliquots (up to 10 possible with 1 residual) and 3 Buffy Coats

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



- Store tubes at room temp.
- Each tube should be labeled with Collection Tube and Site and RAVE ID Labels.

- Collect blood into each EDTA 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.

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

- Centrifuge samples at 1500 x g for 15 minutes at 4°C.

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

- Label clear-capped cryovials with "BUFFY COAT" labels.
- Using a clean transfer pipette, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat from each EDTA tube into its own cryovial.
- Store buffy coat aliquots upright at -80°C until shipment to NCRAD.
- Spin, aliquot, and freeze all plasma and buffy coat aliquots within 2 hours of collection.

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

6.6 Sodium Heparin (Green-Top) Blood Collection Tubes (10 ml) for PBMC

Whole Blood Collection for extraction of PBMC: Sodium Heparin (Green-Top) Blood Collection Tube (10 ml). Two green top tubes are collected at every study visit (both Longitudinal and Biofluid-Focused Arms) obtaining biospecimens.

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

Once drawn, Sodium Heparin tubes MUST be shipped to NCRAD the day of collection via UPS Priority Overnight. This is to ensure the specimen has the most viable cells available at extraction.

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

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

1. Store empty sodium heparin (NapHep) tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use.

2. Place completed Site and RAVE ID label and pre-printed **PBMC** collection tube label on the Sodium Heparin (Green-Top) Blood Collection Tubes (10 ml).

3. Using a blood collection set and a holder, collect blood into the Sodium Heparin (Green-Top) Blood Collection Tubes (10 ml) using your institutions recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

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

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

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

6. Ship both unprocessed Sodium Heparin (Green-Top) Blood Collection tubes *ambient* to NCRAD the day of the participant visit. Please see Section 8.2 for detailed ambient shipping instructions.

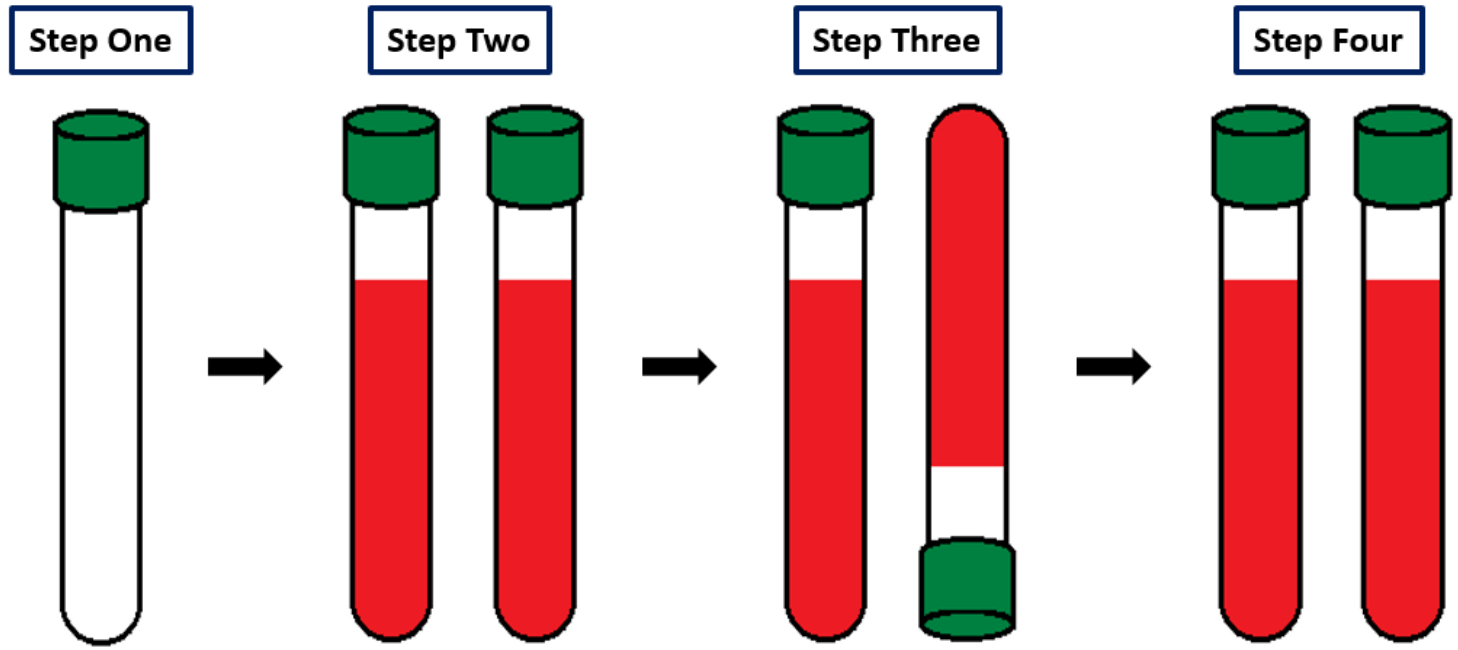
7. Complete Blood Sample and Shipment Notification Form (Appendix B).

8. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu

   Attach the following to the email:

   - Completed Biological Sample and Shipment Notification Form to the email notification. (See Appendix B and/or Appendix C for the NCRAD sample forms)
   - If email is unavailable please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.

PBMC Preparation (10ml Sodium Heparin Green-Top Tube x 2)



- Store tubes at room temp.
- Label tubes with pre-printed Site and RAVE ID and collection tube labels prior to blood draw.

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

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

- Store tubes at room temp. until shipment.
- **Ship ambient same day as blood draw.**

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

6.6 Serum Determination (Red-Top) Tube (10 ml) for Serum

Whole Blood Collection for Isolation of Serum: Serum Determination (Red-Top) Tube (10 ml) (for processing of serum aliquots). One Red-Top tube is collected at every study visit (both Longitudinal and Biofluid-Focused Arms).

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

1. Store empty plain red-top serum blood collection tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use.

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

3. Place completed Site and RAVE ID label and pre-printed **"SERUM"** collection tube label on the red-top serum tube. Place pre-printed "**SERUM**" labels on the (4) 2 ml cryovial tubes with red caps.

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

5. Using a blood collection set and a holder, collect blood into**: Serum Determination (Red-Top) 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 donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into last collection tube.
   d. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.

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

7. Immediately after blood collection, gently invert/mix (180 degree turns) each tube 8-10 times.

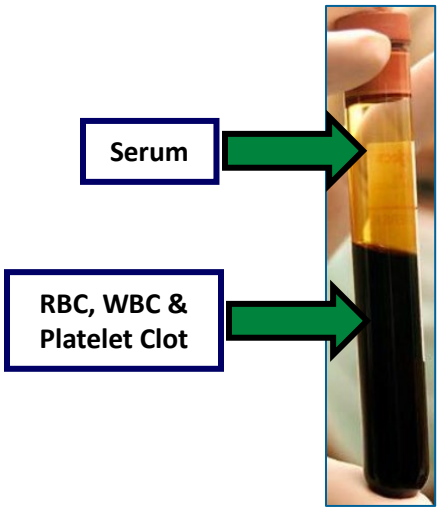
8. **Allow blood to clot at room temperature by placing it upright in a vertical position in a tube rack for 30 minutes.** If sample is not clotted allow it to set up to 60 minutes to clot, checking in 10-minute intervals.

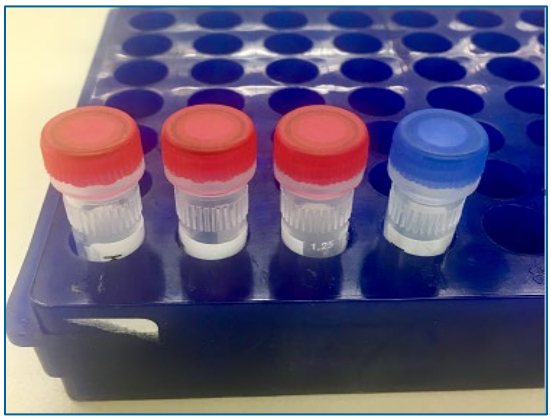
9. After 30 minutes of clotting, centrifuge the collection tube for 15 minutes at 1500 rcf (x g) at 4°C. Serum samples need to be spun, aliquoted, and stored at -80°C within 2 hours of the time of collection. **It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum separation (see worksheet in Appendix**

A to calculate RPM with a particular rotor, or refer to: http://www.sciencegateway.org/tools/rotor.htm).

a. Equivalent rpm for spin at 1500 x g
b. While centrifuging, record the centrifugation start time on the Biological Sample and Shipment Notification Form (Appendix B).

10. Remove the serum, being careful not to disturb the clot at the bottom of the collection tube, by tilting the tube and placing the disposable graduated transfer pipette tip along the lower side of the wall without touching the clotted pellet, so that serum is not contaminated by pellet material. Using a disposable graduated transfer pipette, transfer serum into the pre-labeled cryovials. Aliquot 1.5 ml per cryovial (3 total vials with 1.5 ml in each). The red-top tube should yield, on average, 5 ml of blood serum for a total of 3 aliquot cryovial tubes per participant with 1.5 ml per cryovial tube (note each aliquot tube holds 2ml). Be sure to only place **serum** in cryovials with red lids labeled with the **"SERUM"** label. Place residual serum (<1.5 ml) in the blue-capped cryovial. **If a residual aliquot (<1.5 ml) is created, document the last four digits of the barcode and volume on the Biological Sample and Shipment Notification Form.**



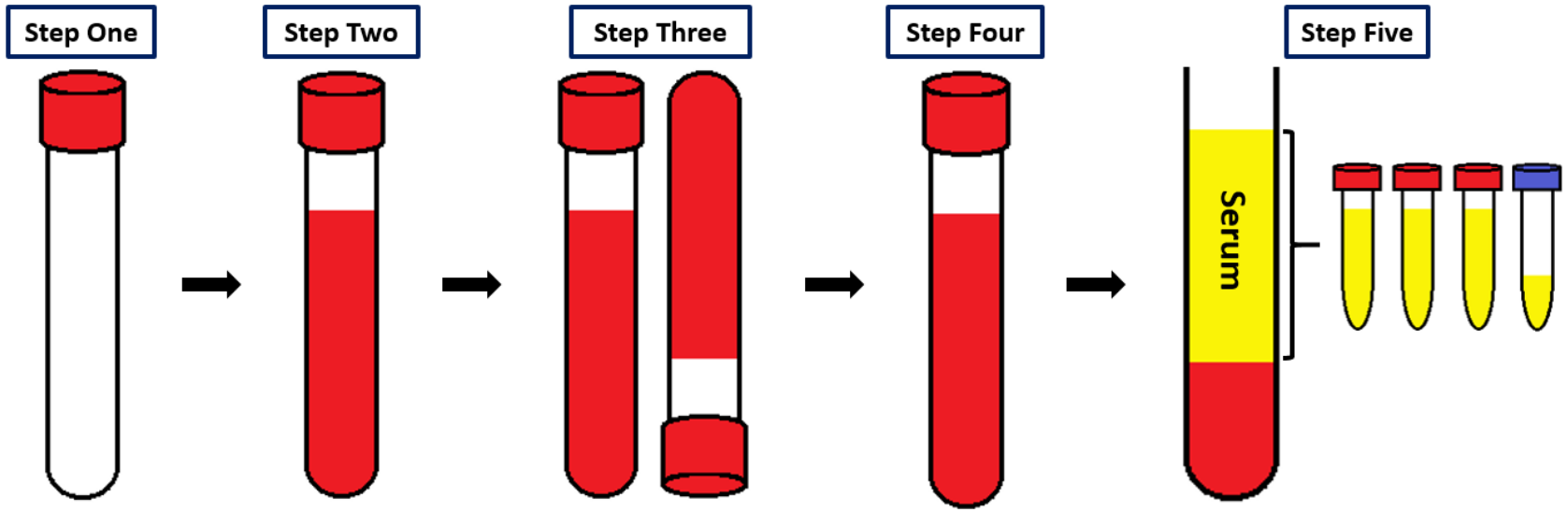


Serum Aliquots (up to 4 possible with residual)

11. Place the labeled cryovials in the 25 cell cryobox and place on pelleted dry ice. Transfer to -80°C Freezer when possible. Store all samples at -80°C until shipped to NCRAD on pelleted dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

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

Serum Preparation (10ml Serum Determination Tube x 1)



Step One
- Store tubes at room temp.
- Each tube should be labeled with Collection Tube and Site and RAVE ID Labels.

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

Step Three
- Immediately after blood draw, invert tube 8-10 times to mix sample.

Step Four
- Allow blood to clot for 30 minutes.
- Within 45 minutes of blood draw, centrifuge samples at 1500 x g for 15 minutes at 4°C.

Step Five
- Label three red-capped cryovials and one blue-capped cryovial with "SERUM" labels.
- Aliquot 1.5 ml into each cryovial. If residual aliquot is created, document specimen number and volume on Sample Form.
- Store serum aliquots upright at -80°C until shipment.
- Spin, aliquot, and freeze aliquots within 2 hours of collection.

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

6.7 PAXgene™ Blood Collection Tube (2.5 ml) for RNA

Whole Blood Collection for Isolation of RNA: two PAXgene™ Blood Collection Tubes for RNA. Two PAXgene™ tubes are collected at every ALLFTD study visit **(both Longitudinal and Biofluid-Focused Arms)**.

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

Draw the PAXgene™ tubes LAST, after all other specimens are collected for the ALLFTD study. The Serum Determination Tube must be the tube drawn immediately BEFORE the PAXgene™ tubes. The Serum Determination Tube draw will ensure that additives within the other collection tubes are not mixed with the PAXgene™ specimen draw.

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

1. Store PAXgene™ Blood Collection Tubes at room temperature 64°F - 77°F (18°C to 25°C) before use.

2. Place completed Site and RAVE ID label and "**RNA**" collection tube label on the PAXgene™ Blood Collection Tubes (2.5 ml) prior to blood draw; no processing is required for these tubes**; the two tubes are to be shipped to NCRAD frozen, without processing at the collection site.**

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

The following techniques shall be used to prevent possible backflow:

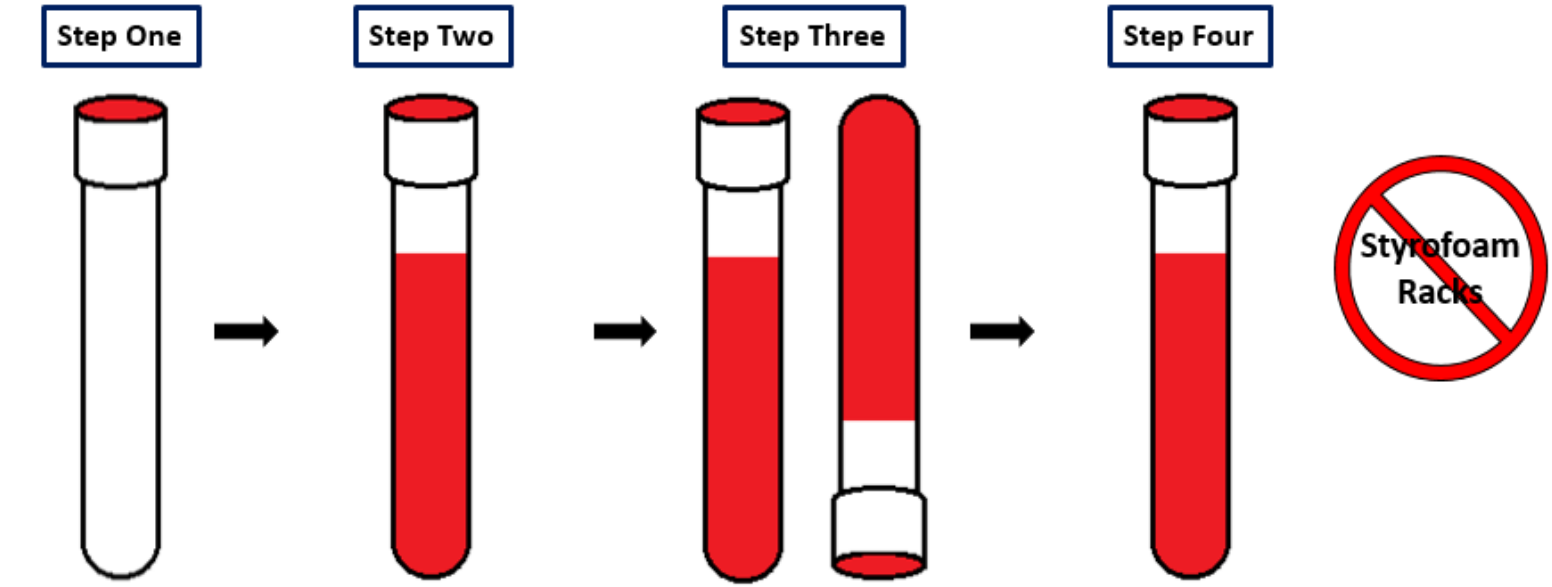
   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into last collection tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The PAXgene™ Blood RNA Tube with its vacuum is designed to draw 2.5ml of blood into the tube. Record total amount of blood drawn into PAXgene™ blood tube(s) within the Biological Sample and Shipment Notification Form.

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

6. Place the PAXgene™ tubes upright in a **WIRE** or **PLASTIC** rack. Transfer to **-80°C Freezer within two hours of the draw**. Record vial location and freezer on batch record. Store all samples at **-80°C until shipped** to NCRAD on dry ice. **Do NOT use a Styrofoam rack. This** will cause the PAXgene™ tubes to crack. Complete remainder of the Biological Sample and Shipment Notification Form (Appendix B).

RNA Preparation (2.5ml PAXgene™ Tube x 2)



- Store tubes at room temp.
- Label tubes with pre-printed subject 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 tube 8-10 times to mix sample.

- Store tubes in wire rack at -80°C until shipment to NCRAD.
- Do not store tube in Styrofoam racks.

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

6.8 Sample Redraws

There may be situations that arise that require a patient sample to be redrawn from certain cycles/visits. At those times, NCRAD study staff will alert site coordinators that a participant sample has failed and should be redrawn. This can happen for several reasons, including insufficient blood at the time the sample was drawn, temperature storage extremes, or even shipping errors.

Redraw kits may vary depending upon the sample(s) that failed and need to be redrawn. Tubes that may be redrawn using the redraw kit include the EDTA (Lavender-Top) Blood Collection Tube (10 ml) and the Sodium Heparin (Green-Top) Blood Collection Tube (10 ml). **Both of these tubes should be sent back to NCRAD ambient and unprocessed.**

Please note: The Sodium Heparin (Green-Top) Blood Collection Tubes (10 ml) (for PBMCs) in the redraw kit should not be collected on a Friday. Only draw blood for these tubes on Monday-Thursday. Always keep in mind holiday closures. Please see: https://ncrad.org/friday_blood_draws.html for a complete list of sample types and how to handle Friday Blood Draws.

Please note: The EDTA (Lavender-Top) Blood Collection Tube (10 ml) may be drawn any day of the week. If an EDTA tube is drawn on a Friday, please hold at room temperature until it can be shipped the following Monday. Samples drawn on Monday-Thursday, can be shipped on the same day as the blood draw.

A sample redraw may occur in one of two ways:

1. The participant travels back to the ALLFTD site and the coordinator redraws the blood and ships it ambient back to NCRAD.

OR

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

Please see Appendix D and Appendix E for Biological Shipping Forms for participants who are provided blood kits for their local physicians.

7.0 Cerebrospinal Fluid Collection

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

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

7.1 Lumbar Puncture Supplies

The lumbar puncture tray contains the following items, which will be used to perform lumbar puncture. Check the dates of expiration: these reflect the expiration date of the lidocaine. Supplies for collection and shipment of CSF are sent to sites in a separate kit from NCRAD.

7.1.1 Lumbar Puncture Tray Components

Quantity	Lumbar Puncture Tray Kit Components
1	Sprotte needle, 24G x 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
1	Lidocaine 1%, 5 ml
1	Povidone-Iodine Topical Solution, 0.75 oz

Sterile, individually packaged 50ml conical tubes are available to sites completing the Lumbar Puncture through the use of the gravitational method. Because not all sites are utilizing this method, the sterile conical tubes must be requested separately from the kit. They are located within the Individual Supply list of the kit request module (Please see Section 5.1).

Do not send blue cap conical tubes used to collect CSF to NCRAD. Discard according to local guidelines. Collection and processing site staff may write on this conical tube for their own reference.

7.2 Setting Up the LP

1. On an overbed table, remove the contents of the LP kit from the outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything wrapped until the person performing the LP is seated and begins examining the participant.

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

3. Touch only the outside of the paper wrapper. When you grab an edge to unfold it, touch only the folded under portions of the outside of the wrapper. Also, don't let the outside of the wrapper touch any part of the inside. If you touch any part of the paper wrapper, or if any non-sterile object or outside of the wrapper touches any part of the inside of the wrapper, discard the kit and start over. If you are in doubt as to whether something touched the inside of the paper wrapper, throw the kit away and start over.

7.3 Maintaining the Sterile Field

1. Keep in mind that there is usually a lot of staff in the room during an LP, and a big part of assisting with the LP is keeping the field sterile—keeping people away from it, and reminding them to be careful around it. If anyone touches the inside of the paper wrapper or any part of the contents of the kit, throw away the kit away and start over. If there is any doubt as to whether someone touched the kit, throw it away and start over. Also, you are the monitor for whether the person performing the LP has broken sterility usually by touching something not sterile with a sterile gloved hand. Feel free to speak up and inform people if need be. Be assertive.

7.4 Tips for Clinicians Performing Lumbar Puncture

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

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

2. Use of a Sprotte 24g atraumatic spinal needle and careful technique are optimal for reducing post-LP headache risk. This Sprotte 24g atraumatic spinal needle is included in the NCRAD LP Tray; additional needles may be ordered upon request. A pencil point spinal needle such as Whitacre 24g, Spinocan 22g or 24g may also be used.
3. Use adequate local anesthesia. Use the 25g 1/2" needle and inject lidocaine to raise a skin wheal. Then, inject lidocaine using the pattern of a square — first the center, and then to all 4 corners. If the participant is thin, do not insert the deep infiltration needle OR the spinal introducer all the way. Use only about 2/3 of their length (to prevent entering the subarachnoid space with anything other than the 24g pencil point spinal needle).

4. Increasing fluid intake immediately after LP is helpful.
5. Be sure to give post-LP care instructions verbally to the participant (see below).

7.5 Post-LP Care Instructions

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

7.5.1 Mild to Moderate headache after a lumbar puncture

- Mild to Moderate headache following lumbar puncture usually resolves within 3-4 days.
- Treatment of Mild to Moderate headache
    - Limit physical activity as much as possible.
    - Oral fluids and caffeine are helpful. Drinking a can of Mountain Dew soft drink (for example) is preferable to coffee, which has some diuretic activity.
    - Tylenol should be used for symptomatic relief. If a participant cannot tolerate Tylenol, ibuprofen should be used. Avoid aspirin. If these do not relieve the headache, Tylenol with codeine or an equivalent could be considered.

7.5.2 Severe headache after a lumbar puncture

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

7.6 Detailed Lumbar Puncture Procedure

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

Place the "**CSF"** Collection and Aliquot Tube Labels on the aliquot tubes (per Section 6.1). Prepare the 17 aliquot tubes provided by NCRAD based on the collection of ≤25 mls of CSF. Additional tubes may be necessary; these tubes may be retrieved from the ALLFTD Supplemental kit provided to each site.

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

2. Perform lumbar puncture using the atraumatic technique.

3. Collect CSF into syringes or sterile conical tube (if a noticeably bloody tap, discard the first 1-2 mls). After the LP has begun and fluid is being collected, take the first 1-2 mls of CSF from the first syringe and place in the CSF labs tube (ORANGE TOP), and send it to the local lab for routine diagnostic tests. **Do not freeze this sample.**

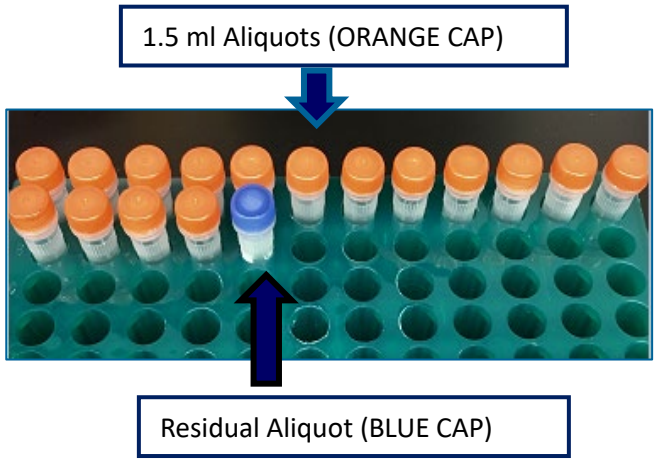
    - Send at room temperature to local clinical lab for basic CSF analysis.
        1. Cell count
        2. Total protein
        3. Glucose
    - *NOTE: Sample must be analyzed within 4 hours of collection.*

4. Collect an additional 23 mls of CSF and transfer to 50 ml conical polypropylene tubes at room temperature. Mix gently by inverting 3-4 times. Record the time of draw (once collection is complete) on the CSF Sample and Shipment Notification Form.

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

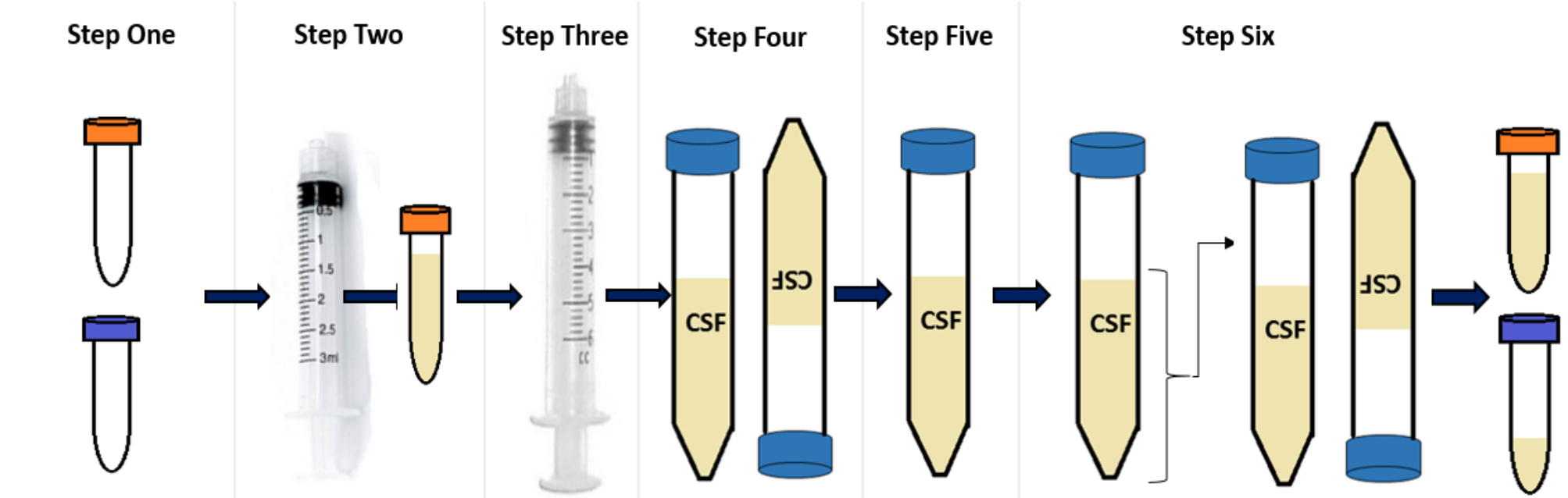
6. Pipette (micropipette preferred) 1.5 ml of supernatant directly into pre-cooled polypropylene CSF collection aliquot tubes. This will yield, on average, 16 aliquot tubes per participant and 1 residual aliquot. (Use more aliquot tubes if needed do not discard any CSF). Seal each aliquot tube with correct cap color. If there is residual amount of CSF remaining (<1.5 ml), please utilize a BLUE cap cryovial to indicate that this aliquot has low volume.

- If at least 20 mls are able to be sent to NCRAD, the remaining 3 mls can be kept locally. If there is no local repository, NCRAD will accept all aliquots.



7. Within 60 minutes of CSF collection, freeze aliquots immediately on pelleted dry ice and then store at **-80°C** or ship on pelleted dry ice in a shipping container. Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the RAVE database.

CSF Preparation (20-25 mL in Syringes)



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

- Collect CSF into the 3mL luer lock syringe.
- Dispense 1-2mL in ORANGE cap cryovial.
- Send to local lab for testing

- Collect CSF into the 6mL luer lock syringe.
- Collect 20-23 mL.

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

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

- Using a clean transfer pipette, transfer all CSF into a 50 mL conical tube leaving the pellet in the bottom and mix gently by inverting 3-4 times.
- Aliquot 1.5 ml into 15 ORANGE CAP CRYOVIALS. Aliquot residual mL in last BLUE CAP CRYOVIAL
- Store CSF aliquots at -80°C until shipment.
- Remaining 1-3 mL may stay at local lab for researcher or submit with other cryovials to NCRAD.

8.0 Packaging & Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your University, training and certification is available through the CITI training site (Course titled "Shipping and Transport of Regulated Biological Materials" at https://www.citiprogram.org/).

Sample Type	Study Visits Collecting Biospecimens	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes sent to NCRAD	Ship
Whole blood (Lavender-Top EDTA) for isolation of plasma & buffy coat (for DNA extraction)	**All Cycles**	**3**	**3**	**N/A**	**N/A**
	All Cycles	10 (9 Lavender Cap, 1 Blue Cap Cryovial)	1.5 ml plasma aliquots per 2.0 ml cryovial	8-10	Frozen
	All Cycles	3	1 ml buffy coat aliquot per 2.0 ml cryovial	3	Frozen
Whole blood (Green-Top Sodium Heparin) for PBMC isolation	**All Cycles**	**2**	**N/A**	**2**	**Ambient**
Whole blood (Red-Top Serum) for isolation of serum	**All Cycles**	**1**	**1**	**N/A**	**N/A**
	All Cycles	4 (3 Red Cap, 1 Blue Cap Cryovial)	1.5 ml Serum Aliquots Per 2.0 ml cryovial	3-4	Frozen
Whole blood (PAXgene™) for RNA isolation	**All Cycles**	**2**	**N/A**	**2**	**Frozen**
CSF	***Optional for all cycles**	**17 (16 Orange Cap, 1 Blue Cap Cryovial)**	1.5 ml CSF aliquots per 2 ml cryovials	**13-17**	**Frozen**

8.1 Frozen Packaging Instructions

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

The most important issue for shipping is to maintain the temperature of the samples. The frozen samples must never thaw; not even the outside of the tubes should be allowed to defrost. This is best accomplished by making sure the Styrofoam container is filled completely with pelleted dry ice

Large Frozen Shipper:

** 45 lbs of dry ice pellets

AND

- Fits up to 8 x 25-cell cryoboxes

Small Frozen Shipper:

**10 lbs of dry ice pellets

AND

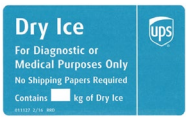
- Fits up to 3 x 25-cell cryoboxes

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

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

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

- The primary receptacle (PAXgene™ RNA tubes or 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 (cryovial box containing the frozen cryovials, or PAXgene™ RNA tubes) and the secondary packaging (biohazard bag). 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
  - ✓ Class 9 label including UN 1845, and net weight of pelleted dry ice contained



1. If possible, hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off. If storage in a -80°C freezer until UPS pick-up is not possible, package samples no more than 4 hours before the expected pick-up time.

2. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu. Attach the following to the email:

   a. Completed Biological Sample and Shipment Notification Form to the email notification. (See Appendix B and/or Appendix C for 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.

3. Place all frozen labeled 1.5 ml aliquots of plasma, buffy coat, and serum in the one 25-slot cryobox.

   a. Each cryobox holds up to 25 cryovials and there will be a maximum of 17 cryovials (10 plasma, 4 serum, and 3 buffy coat) per blood draw (see next page).

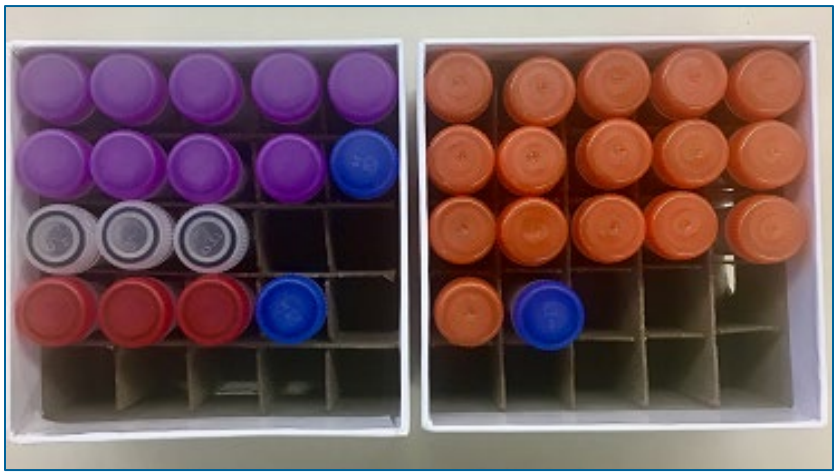
4. If CSF is drawn at that time point, include the CSF aliquots in a separate 25-count cryobox in the same batch shipment (see next page).

5. A batch shipment should contain all of the specimens from the same patient, per time point. For example, if blood and CSF were collected at the same time point for a participant, two 25-count cryoboxes should be included in the same batch shipment. They will be placed in separate small biohazard bags.

- **Batch shipping should be performed quarterly or when 8 cryoboxes of samples have been obtained.**

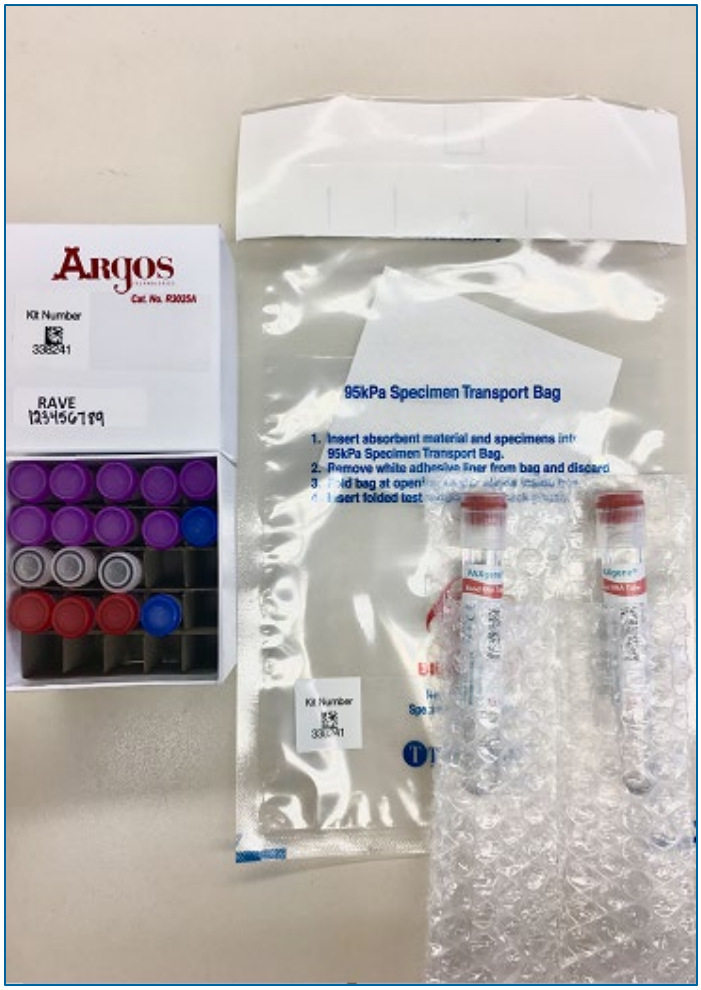
  - Example #1: 4 participants with blood AND CSF collected (8 total cryoboxes for 4 participants)
  - Example #2: 8 participants with ONLY blood collected (8 total cryoboxes for 8 participants)
  - Example #3: 2 participants with blood AND CSF collected (4 total cryoboxes for 2 participants) and 4 participants with ONLY blood collected (4 total cryoboxes for 4 participants)

25-Slot Cryoboxes Containing Biospecimens from One Blood kit and One CSF kit



Blood kit (left) and CSF kit (right) Prepped for Batch Shipping

***Note: Blood and CSF kits will have different kit numbers, but the same RAVE IDs**



6. Label the outside of each cryobox with the kit number label (shown above) and the RAVE ID. Please place the cryoboxes containing blood derivatives in one small biohazard bag. Label the outside of the small biohazard bag with a kit number label as well (shown above).

7. **If collected, place the cryoboxes containing CSF derivatives for the patient in a separate small biohazard bag.** Label the outside of this second small biohazard bag with the CSF kit label.

8. Insert PAXgene™ tubes into the bubble slots and place within the small biohazard bag that contains the cryobox with the blood samples. Insert only PAXgene™ tubes that match the patient numbers and time points of the blood samples in the cryoboxes (e.g. do not insert extra PAXgene™ tubes from other patients).

9. As the cryoboxes and PAXgene™ tubes are placed in the small clear plastic biohazard bag, do NOT remove the absorbent material found in the bag. Seal according to the instructions on the bag. The kit number label should have been placed on each cardboard cryobox prior to inserting into the biohazard bag. A kit number label should also have been placed on the outside of the biohazard bag. Please note that if you are shipping blood and CSF samples for 1 participant, the kit numbers will be different on each small biohazard bag.

Cryobox and PAXgene™ tubes placed in clear biohazard bags

Packaged Blood kit:

Two PAXgene™ tubes inserted in bubble wrap sleeves.

One 25-count cryobox with plasma, serum, buffy coats, and residual aliquots

Packaged CSF kit:

One 25-count cryobox with CSF and residual aliquots

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

11. Place the biohazard bags into the provided Styrofoam-lined shipping container on top of the pelleted dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container. **A maximum of 8 cryoboxes may be sent in each large shipper or a maximum of 3 cryoboxes in each small shipper.**

12. Fully cover the biohazard bags containing the cryoboxes and PAXgene™ tubes with approximately 2 inches of pelleted dry ice.

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

14. 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.

15. 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.

16. 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.

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

18. 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.

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

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

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

For frozen shipments, include no more than eight cryovial boxes (separated by patient within biohazard bags) and 8 bubble-wrap sleeves (2 slots each) 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, buffy coat, serum, CSF, and frozen unprocessed PAXgene™ RNA tubes must be shipped to NCRAD as outlined above.

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

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

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

8.2 Ambient Packaging Instructions

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

For ambient Sodium Heparin (Green-Top) Blood Collection Tube (2 x 10 ml) shipments, include no more than two tubes per shipping container. The ambient PBMC samples must be shipped the day of blood draw. The labeled, unprocessed, sodium heparin PBMC tube will be shipped to NCRAD as outlined below.

IMPORTANT!
AMBIENT SAMPLES MUST BE SHIPPED MONDAY-THURSDAY ONLY! **Do NOT draw blood for ambient shipments on Fridays!**

Ambient Sodium Heparin (Green-Top) Blood Collection Tube (2 x 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.

***** Ambient 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 (small biohazard bag) must be leak proof.
- Absorbent material must be placed between the primary receptacle and the secondary packaging (small biohazard bag). 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

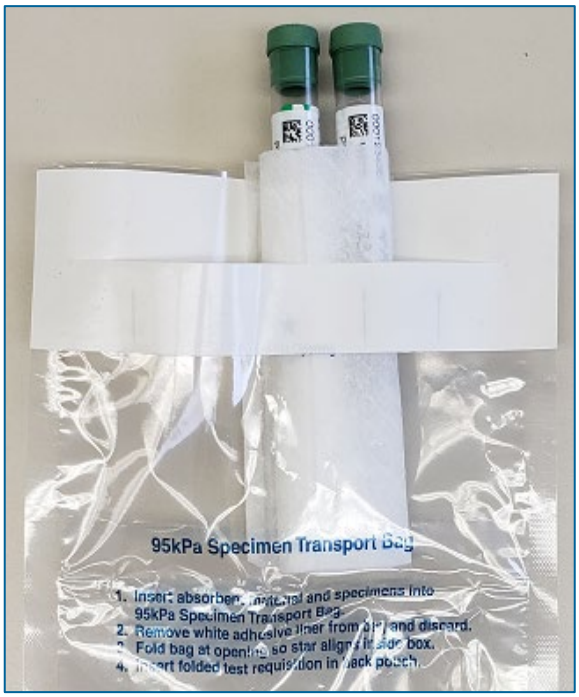
1. Place refrigerant pack in freezer 24 hours prior to shipment.

2. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu
   a. Complete and attach the Biological Sample and Shipment Notification Form to the email. (See Appendix B for an example of the form)

3. Place filled and labeled sodium heparin tubes within the slots in the absorbent pad provided, and place into the plastic biohazard bag with absorbent sheet.

4. Place Kit Number Label on outside of the biohazard bag.

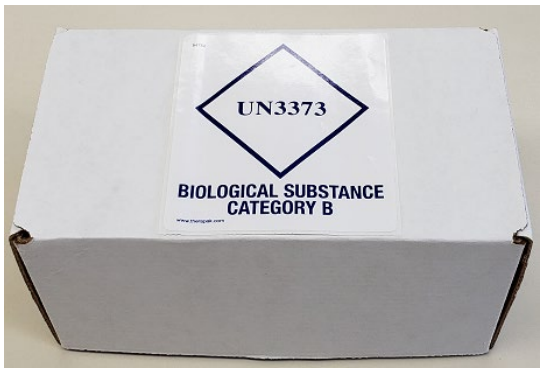
5. Remove as much air as possible from the plastic biohazard bag and seal the bag according to the directions printed on the bag.



6. Place the refrigerant pack into the cooler on top of the filled biohazard bag.

7. Place the lid onto the cooler.

8. Place an extra copy of the emailed “Biological Sample and Shipment Notification Form” within the shipping box along with a list of contents form.



9. Close shipping box. Label the outside of the cardboard box with the enclosed UN3373 (Biological Substance Category B) label.

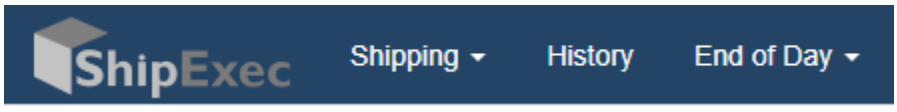
10. Place UPS return airbill on the sealed UPS Laboratory Pak.

11. Use UPS tracking to ensure the delivery occurs as scheduled and is received by NCRAD.

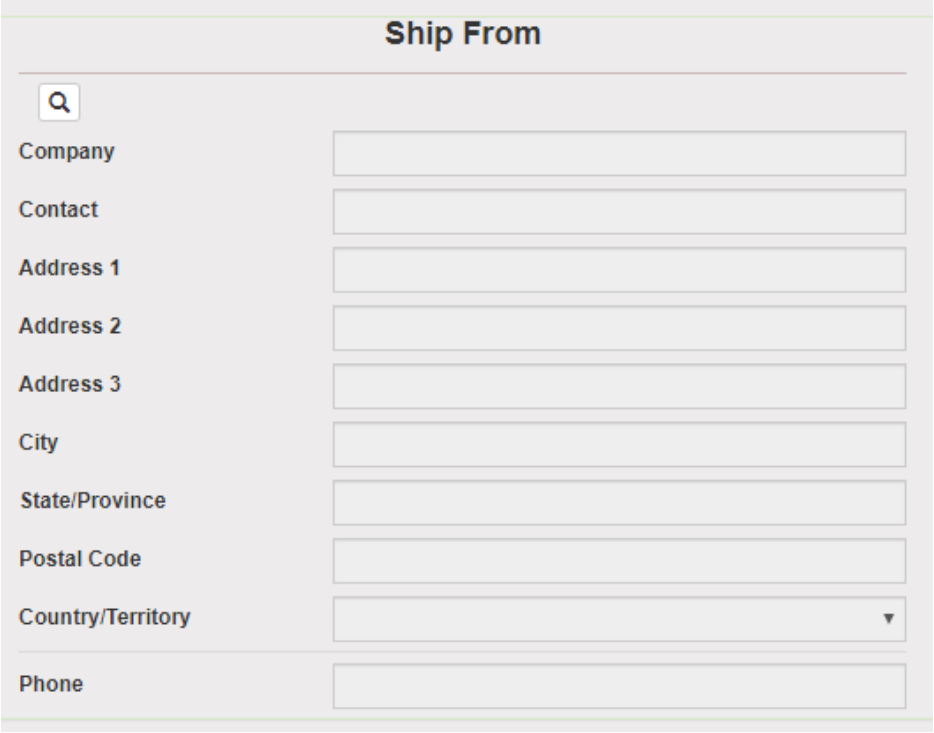
8.3 Ambient and Frozen Shipping Instructions

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



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



a. Search by Company (site), Contact (name), or Address 1 (first line of your site's street address). Click Search.
b. Click Select to the left of the correct contact information.

4. Verify that both the shipping information AND study reference are correct for this shipment.
    a. If wrong study contact or study reference, click Reset in the bottom right of the screen to research for the correct information.

5. Enter Package Information
    a. 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 and need to reenter these values.
    c. Click Ship in the bottom right of the page when complete.

6. 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.

7. Fold airbill, and place inside plastic UPS sleeve.

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

9. 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.

8.4 International Shipments: Canada to U.S.A

1. All international shipments to be made between Canada and the USA will utilize the same packing requirements as specified in Section 8.1 (Frozen Packaging Instructions), Section 8.2 (Ambient Packaging Instructions), and Section 8.3 (Ambient and Frozen Shipping Instructions).

9.0 Data Queries and Reconciliation

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

The ALLFTD iMedidata RAVE data collection team will be collaborating with NCRAD to reconcile information captured in the database with information from the samples received and logged at NCRAD. Information that appears incorrect in the iMedidata RAVE database will be queried through the standard system. Additional discrepancies that may be unrelated to data entry will be resolved with the Principal Investigator in a separate follow up communication. If applicable, a non-conformance report will be provided to sites on a monthly basis.

Data queries or discrepancies with samples shipped and received at NCRAD may result from:

- Missing samples
- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Biological Sample and Shipment Notification Form and logged at NCRAD compared to information entered into the iMedidata RAVE database.
- Samples that are frozen and stored longer than one quarter at the site
- Use of an incorrect Biological or CSF Sample and Shipment Notification Form

10.0 Appendices

Appendix A: Rate of Centrifuge Worksheet

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

Submitter Information

Name: **Site:**

Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

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

Calculating RPM from G-Force:

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

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

Comments:

Please send this form to NCRAD Study Coordinator

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

Appendix B: Blood Sample and Shipment Notification Form

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

To: Kelley Faber | Email: alzstudy@iu.edu | FAX: 317-278-1100 | Phone: 1-800-526-2839

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

Phone:_____ **Email:**_____ **Site #:**_____

Study: ALLFTD Longitudinal ☐ ALLFTD Biofluid ☐ ALLFTD/4RTNI-2 Dual-enrollment ☐

RAVE ID:_____ **RAVE Cycle:**_____

Sex: ☐ M ☐ F **Year of Birth:** _____

Kit #: KIT BARCODE

Blood Collection:

Date of Draw: _____ [MMDDYY]	Time of Draw: _____ [HHMM]
Date participant last ate: _____ [MMDDYY]	Time participant last ate: _____ [HHMM]

PBMC (NaHep Tubes)

#1	Last four digits of PBMC barcode:	_____	Original volume drawn:	_____ml
#2	Last four digits of PBMC barcode:	_____	Original volume drawn:	_____ml

RNA (PAXgene™ Tubes)

Original volume drawn (2 x 2.5 ml PAXgene™ tubes):	Tube 1: _____ ml Tube 2: _____ ml
PAXgene™ tubes Time frozen:	_____ [HHMM]

Storage temperature of freezer: _____°C

Blood Processing:

Plasma & Buffy Coat (Lavender-Top) Tube (10 mL)	
Time spin started:	_____ [HHMM]
Duration of centrifugation:	_____ Minutes
Temp of centrifuge: ____ °C Rate of centrifuge: _____ x g	
Time aliquoted:	_____ [HHMM]
Number of 1.5 mL plasma aliquots created (lavender cap, up to 9):	_____
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	
Time plasma and buffy coat aliquots frozen:	_____ [HHMM]
Serum (Red-Top) Tube (10 mL)	
Time spin started	_____ [HHMM]
Duration of centrifugation:	_____ Minutes
Temp of centrifuge: ____ °C Rate of centrifuge: _____ x g	
Time aliquoted:	_____ [HHMM]
Number of 1.5 mL serum aliquots created (red cap, up to 3):	_____
If applicable, volume of residual serum aliquot (less than 1.5 mL in blue cap):	_____ mL
If applicable, specimen number of residual serum aliquot (last four digits):	_____
Time serum aliquots frozen:	_____ [HHMM]

NOTES:_____

Appendix C: CSF Sample and Shipment Notification Form

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

To: Kelley Faber Email: alzstudy@iu.edu FAX: 317-278-1100 Phone: 1-800-526-2839

From:_____ **FedEx tracking #:**_____

Phone:_____ **Email:**_____

Site #:_____

Study: ALLFTD Longitudinal ☐ ALLFTD Biofluid ☐

RAVE ID: _____ **RAVE Cycle:** _____

Sex: ☐ M ☐ F **Year of Birth:** _____

Kit #: KIT BARCODE

CSF Collection:

1. Date of Draw: _____ [MMDDYY]	2. Time of Draw: _____ [HHMM]
3. Date participant last ate: _____ [MMDDYY]	4. Time participant last ate: _____ [HHMM]
Collection Process: ☐ Gravitational **OR** ☐ Pull	

CSF Processing:

Time spin started:	_____ [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 CSF aliquots created (up to 15 total): **(Orange cap cryovials):**	_____
If applicable, volume of residual CSF aliquot (less than 1.5 mL): **(Blue cap cryovials):**	_____ mL
If applicable, specimen number of residual aliquot tube: **(Last four digits)**	_____
Time frozen:	_____ [HHMM]
Storage temperature of freezer:	_____°C
NOTES: _____	

Appendix D: Green Top-Sodium Heparin Redraw/Take Home Sample Form

TO BLOOD DRAWING PERSONNEL

This blood sample is for a study sponsored by the National Institute of Health (NIH). Samples are housed at Indiana University School of Medicine. It will need to be shipped to the address below. Please use the enclosed pre-addressed UPS Clinical Pak.

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

The kit provided contains collection tubes with which to obtain blood from the individual for research purposes. Each kit contains 2 green-topped tubes and all necessary shipping supplies.

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

Instructions for drawing and shipping blood samples:

1. Place refrigerant pack in freezer 24 hours prior to shipment.
2. Fill **GREEN TUBES** completely, if possible.
3. Invert (do not shake) tube eight to ten times after drawing blood to thoroughly mix additive with sample.
4. **Enclose this form in shipment with samples**. Place green tubes in biohazard bag and seal, then place bag and gel pack in the Styrofoam container and close.
5. Ship samples by **UPS** immediately after drawing. Use the enclosed, pre-paid UPS mailer. There will be no cost to you or the patient for the shipping.

KIT NUMBER (RECORDED ON LABEL): _____

RAVE IDENTIFICATION NUMBER (RECORDED ON LABEL): _____

RAVE CYCLE NUMBER: _____

STUDY SITE ID (RECORDED ON LABEL): _____

DATE BLOOD WAS DRAWN: _____

DONOR YEAR OF BIRTH: _____

DONOR SEX: _____

Appendix E: Lavender Top-EDTA Redraw/Take Home Sample Form

TO BLOOD DRAWING PERSONNEL

This blood sample is for a study sponsored by the National Institute of Health (NIH). Samples are housed at Indiana University School of Medicine. It will need to be shipped to the address below. Please use the enclosed pre-addressed UPS Clinical Pak.

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

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

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

Instructions for drawing and shipping blood samples:

1. Place refrigerant pack in freezer 24 hours prior to shipment.
2. Fill **LAVENDER TUBES** completely, if possible.
3. Invert (do not shake) tube eight to ten times after drawing blood to thoroughly mix additive with sample.
4. **Enclose this form in shipment with samples**. Place lavender tubes in biohazard bag and seal, then place bag and gel pack in the Styrofoam container and close.
5. Ship samples by **UPS** immediately after drawing. Use the enclosed, pre-paid UPS mailer. There will be no cost to you or the patient for the shipping.

KIT NUMBER (RECORDED ON LABEL): _____

RAVE IDENTIFICATION NUMBER (RECORDED ON LABEL): _____

RAVE CYCLE NUMBER: _____

STUDY SITE ID (RECORDED ON LABEL): _____

DATE BLOOD WAS DRAWN: _____

DONOR YEAR OF BIRTH: _____

DONOR SEX: _____

Appendix F: Low Fat Diet Menu Suggestions

Foods to avoid prior to Lumbar Puncture and/or blood collection:

Avoid: *All fats and nuts such as:*

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

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

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

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

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

Appendix G: Biofluid-Focused Arm Collection

All sample forms must be completed for Biofluid-Focused Arm participants, similarly to Longitudinal Arm participants.

Collection Alternatives:

During a biofluid-focused arm collection **only**, a site may not be able to collect all tubes listed in the ALLFTD protocol. When this occurs, please follow the steps below:

The standard order of specimen collection is:

1. EDTA (Lavender-Top) for Plasma and Buffy Coat (3 x 10ml tubes)
2. Sodium Heparin (Green-Top) for PBMC (2 x 10ml tubes)
3. Serum Determination (Red-Top) for Serum (1 x 10ml tube)
4. PAXgene™ for RNA (2 x 2.5ml tube)

If a site is unable to collect and process all 62.5ml of blood at the biofluid arm visit, please maintain the order of draw and collect as many tubes as possible.

If a site is unable to collect and ship the PBMC tubes the same day of collection, please collect in this order:

1. EDTA (Lavender-Top) for Plasma and Buffy Coat
2. Serum Determination (Red-Top) for Serum
3. PAXgene™ for RNA

Please indicate on the sample form that a sample was not collected by checking the N/A box next to that sample type.

Minimum amounts: Blood Collection

The minimum amount of blood for plasma/buffy coat that can be collected is 2 10ml lavender-top EDTA tubes. If 2 10ml lavender-top EDTA tubes are drawn, please continue drawing in the standard order.

- The minimum amount of blood for PBMCs that can be collected is 1 10ml green-top Sodium Heparin tube. If 1 10ml green-top Sodium Heparin tube is drawn, please continue drawing in the standard order.
  - The minimum amount of blood for serum that can be collected is 1 10ml red-top serum determination tube. If 1 10ml red-top serum determination tube is drawn, please continue drawing in the standard order.
    - The minimum about of blood for RNA that can be collected is 2 2.5ml PAXgene™ tubes. Once PAXgene™ tubes are drawn, the ALLFTD collection is complete.

Minimum amounts: CSF Collection

The minimum amount of CSF that can be collected for the biofluid-focused arm is 8 mls.