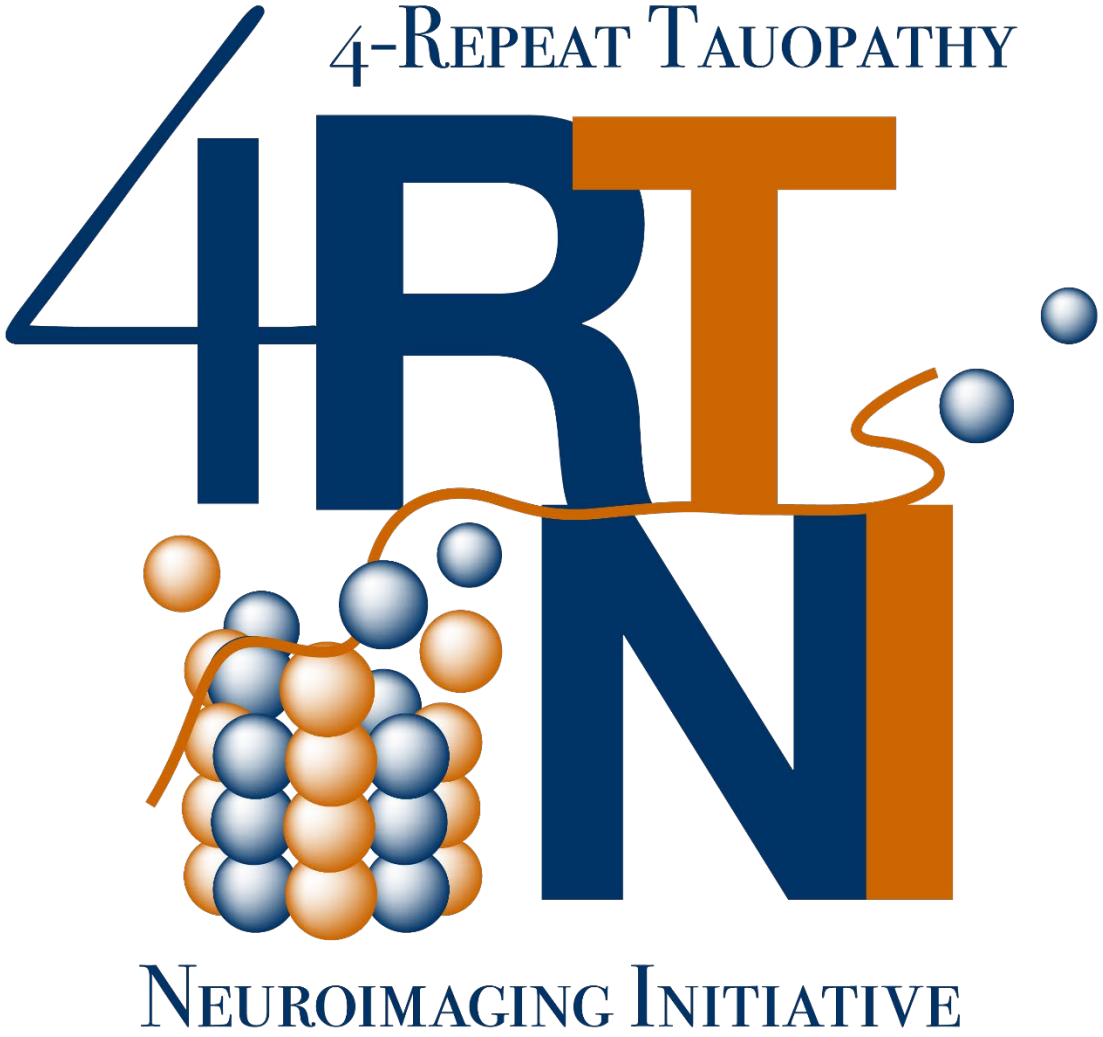
4RTNI-2 Manual of Procedures Update: Version 05.2025

Section	Change
Document Footer	Updated to “Version (May 2025)”
Throughout Document	Minor changes made to phrasing and wording. No alterations to procedures were made.
3.1	Updated contact information



Manual of Procedures

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

4RTNI-2

Biospecimen Collection, Processing, and Shipment Manual

Table of Contents

1.0 Abbreviations

4RTNI-2	4 Repeat Tauopathy Neuroimaging Initiative – Project 2
CSF	Cerebrospinal Fluid
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
LP	Lumbar Puncture
NCATS	National Center for Advancing Translational Sciences
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
NINDS	National Institute of Neurological Disorders and Stroke
PBMC	Peripheral Blood Mononuclear Cell
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

2.0 PURPOSE

The purpose of this manual is to provide 4RTNI-2 staff (PIs, study coordinators, and the sample collection and processing teams) at the various study sites with instructions for collection and submission of biological samples for the 4RTNI-2 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 study visits:

- Plasma
- Buffy Coat (for DNA extraction)
- PBMC
- Serum
- RNA
- CSF (select patient subset only)

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 4RTNI-2 protocol.

3.0 NCRAD Information

3.1 NCRAD Contacts

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

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

General NCRAD Contact Information
Phone: 1-800-526-2839 or 317-278-8413
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: www.ncrad.org
4RTNI-2 Study Specific Webpage: https://ncrad.org/coordinate-studies/4rtni

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

Sample Shipment Mailing Address
4RTNI-2 at NCRAD
Indiana University School of Medicine
351 W. 10th St. TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

3.2 Hours of Operation

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

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

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

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

3.3 Holiday Schedules

- Please note 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.**

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 19th	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 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.

4.0 4RTNI-2 Laboratory Collection

4.1 Site Required Equipment

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

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquet
- Alcohol Prep Pad
- Gauze Pad
- Bandage
- Butterfly needles and hub
- Microcentrifuge tube rack
- Sharps bin and lid
- Wet ice bucket (for CSF only)
- Wet ice (for CSF only)

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

- Centrifuge capable of ≥ 2,000 rcf (2,000 x g) with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:

- Dry ice pellets (approximately 30-45 lbs per shipment)

4.2 Biospecimens Sent to NCRAD

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

	Cycle 1, Baseline	Cycle 2, Visit 2	Cycle 3, Visit 3	Cycle 4, Visit 4
DNA (Buffy Coat)	X	X	X	X
Plasma	X	X	X	X
PBMC	X			
Serum	X			
RNA	X			
CSF	X	X*	X	X

*Collected at visit 2 only if it is a termination visit

Whole blood will be collected in up to four different collection tubes (lavender-top EDTA tube, green-top sodium heparin tube, 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.

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 Biospecimens Collection Charts

4.3.1 Blood Collection: Baseline/Cycle 1

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	**3**	**3**	**N/A**	**N/A**
	PLASMA: 2 ml cryovials with lavender caps (residual volume placed in 2 ml cryovial with blue cap)	31 (30 Lavender Cap, 1 Blue Cap Cryovial)	0.5 ml plasma aliquots per 2.0 ml cryovial	24-31	Frozen
	BUFFY COAT: 2 ml cryovial with a clear cap	3	1 ml buffy coat aliquot per 2.0 ml cryovial	2-3	Frozen
Whole blood for PBMC isolation	**Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)**	**2**	**N/A**	**2**	**Ambient**
Whole blood for isolation of serum	**Serum Determination (Red-Top) Blood Collection Tube (10 ml)**	**1**	**1**	**N/A**	**N/A**
	Serum: 2 ml cryovials with red caps (residual volume placed in 2.0 ml cryovial with blue cap)	11 (10 Red Cap, 1 Blue Cap Cryovial)	0.5 ml Serum Aliquots Per 2.0 ml cryovial	8-11	Frozen
Whole blood for RNA isolation	**PAXgene™ Blood Collection Tube (2.5 ml)**	**3**	**N/A**	**3**	**Frozen**

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

4.3.2 Blood Collection: Visit 2-4/Cycles 2-4

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	**2**	**2**	**N/A**	**N/A**
	PLASMA: 2 ml cryovials with lavender caps (residual volume placed in 2 ml cryovial with blue cap)	21 (20 Lavender Cap, 1 Blue Cap Cryovial)	0.5 ml plasma aliquots per 2.0 ml cryovial	16-21	Frozen
	BUFFY COAT: 2 ml cryovial with a clear cap	2	1 ml buffy coat aliquot per 2.0 ml cryovial	2	Frozen

4.3.3 Cerebrospinal Fluid

Sample Type	Tube Type	Study Visit Collecting Biospecimens	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
CSF	**50 ml screw top centrifuge tubes with blue caps**	**Cycle 1, Cycles 3 & 4 (Collect at Cycle 2 if Termination Visit)**	**2**	**N/A**	**N/A**	**N/A**
	2 ml cryovial with clear cap	Cycle 1, Cycles 3 & 4 **(Collect at Cycle 2 if Termination Visit)**	20	0.5 ml CSF aliquots per 2 ml cryovials	20	frozen
	2 ml cryovial with orange cap (residual volume placed in 2 ml cryovials with blue caps)	Cycle 1, Cycles 3 & 4 **(Collect at Cycle 2 if Termination Visit)**	13 (12 Orange Cap, 1 Blue Cap Cryovial)	1.0 ml CSF aliquots per 2 ml cryovials	10-13	frozen
	2 ml cryovial with orange cap	Cycle 1, Cycles 3 & 4 **(Collect at Cycle 2 if Termination Visit)**	1	1.0-2.0 ml CSF aliquots per 2 ml cryovials	0	To local lab

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.

5.0 Specimen Collection Kits, Shipping Kits and Supplies

Research specimen collection kits as well as clinical lab supplies (except dry ice and equipment supplies listed above) will be provided by NCRAD. These materials include blood tubes, lumbar puncture trays (when applicable), boxes for plasma/buffy coat/CSF aliquots, 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 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.*

4RTNI-2 Cycle 1, Baseline Blood Kit

Quantity	4RTNI-2 Cycle 1, Baseline 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)
3	PAXgene™ Blood Collection Tube (2.5 ml)
30	Cryovial tube (2 ml) with lavender cap
10	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
54	Pre-printed labels for blood collection and aliquot tubes
5	Pre-printed labels with kit number
10	Labels for handwritten Site and RAVE ID
1	Cryovial tube box (holds up to 81 cryovials)
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 6 tube absorbent pouch UN3373 Biological Substance Category B label List of contents card FedEx return airbill FedEx Clinic Pak

4RTNI-2 Longitudinal Blood Kit

Quantity	4RTNI-2 Longitudinal Blood Kit Components
2	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
20	Cryovial tube (2 ml) with lavender cap

2	Cryovial tube (2 ml) with clear cap
1	Cryovial tube (2 ml) with blue cap
2	Disposable graduated transfer pipette
24	Pre-printed labels for blood collection and aliquot tubes
2	Pre-printed labels with kit number
3	Labels for handwritten Site and RAVE ID
1	Cryovial tube box (holds up to 81 cryovials)

4RTNI-2 CSF Kit

Quantity	NCRAD CSF Kit Components
20	Cryovial tube (2 ml) with clear cap
13	Cryovial tube (2 ml) with orange cap
1	Cryovial tube (2 ml) with blue cap
1	Lumbar Puncture tray
2	Screw-top conical tube with blue cap (50 ml)
4	Disposable graduated transfer pipette
35	Pre-printed labels for blood collection and aliquot tube
4	Pre-printed labels with kit number

Ambient Shipping Supply Kit

1	Shipping Supplies for ambient shipment: Plastic biohazard bag with absorbent sheet Small IATA shipping box with insulated cooler Small refrigerant pack Aqui-Pak 6 tube absorbent pouch UN3373 Biological Substance Category B label List of contents card FedEx return airbill FedEx Clinic Pak

Frozen Shipping Supply Kit

Quantity	Frozen Shipping Kit Components
4	Plastic Biohazard bag with absorbent sheet
1	FedEx return airbill and pouch
1	Shipping box/Styrofoam container
1	Warning label packet with dry ice sticker
12	Bubble wrap pouch

Green Top-Sodium Heparin Tube Redraw/Take Home Kit

Quantity	Ambient Shipping Supply Components
2	Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)
2	Pre-printed labels for blood collection tube
2	Pre-printed labels with kit number
3	RAVE ID labels
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 6 tube absorbent pouch UN3373 Biological Substance Category B label List of contents card FedEx return airbill FedEx Clinic Pak

Lavender Top-EDTA Tube Redraw/Take Home Kit

Quantity	Ambient Shipping Supply Components
1	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
1	Pre-printed labels for blood collection tube
2	Pre-printed labels with kit number
2	RAVE ID labels
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 6 tube absorbent pouch UN3373 Biological Substance Category B label List of contents card FedEx return airbill FedEx Clinic Pak

Supplemental Supply Kit

Quantity	4RTNI-2 Supplemental Kit Components
3	Cryovial tube box (holds up to 81 cryovials)
4	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
5	FedEx return airbill

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)
10	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

Individual Supplies

Available upon request within the kit request module.

Quantities	Item
5, 10	PAXgene™ slotted absorbent pad
5, 10	Microcentrifuge tube box (holds up to 81 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	FedEx return airbill
5, 10	Small canister (95kPa Certified)
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
15, 30	Screw-top conical tube with blue cap (50 ml)
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

5.2 Kit Supply to Study Sites

Each individual site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits. Please go to: http://kits.iu.edu/4rtni-2 to request additional kits and follow the prompts to request the desired supplies. Options include ordering specific number of kits or individual supplies.

Please allow **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.

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

1. **EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Buffy Coat and Plasma (X3)**
2. **Sodium Heparin (Green-Top) Blood Collection Tube (10 ml) for PBMC (X2)**
3. **Serum Determination Tube (Red Top) for Serum**
4. **PAXgene™ Blood Collection Tubes for RNA (X3)**

At longitudinal visits, ONLY draw the following:

1. **EDTA (Lavender-Top) Blood Collection tube (10 ml) for Buffy Coat and Plasma (X2)**

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

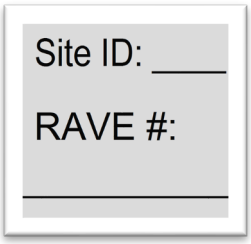
6.1 Labeling Samples

Each kit is supplied with labels for the specimens to be shipped to NCRAD.

Label Type Summary

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

The **Kit Number Labels** do not indicate a specimen type. Place one Kit Number Label within the designated location on the "Biological Sample and Shipment Notification Form" and the "CSF Sample and Shipment Notification Form", if collected. Place the other Kit Number Labels on the cardboard cryobox, biohazard bag, and on the lid of the shipping canisters (Sodium Heparin tubes). See Section 8.0 for further instructions.

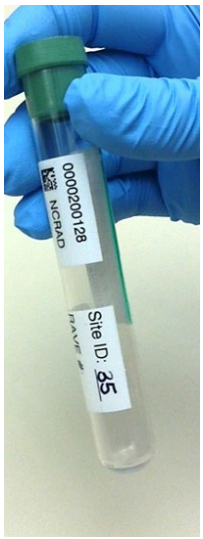


Place one **Site and RAVE ID Label** on each blood collection tube (EDTA, Sodium Heparin, Serum, and PAXgene™). Do not send blue cap conical tubes used to collect CSF to NCRAD. Collection and processing site staff may write on this conical tube for their own reference. The blue cap conical tubes will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

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

The Collection and 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

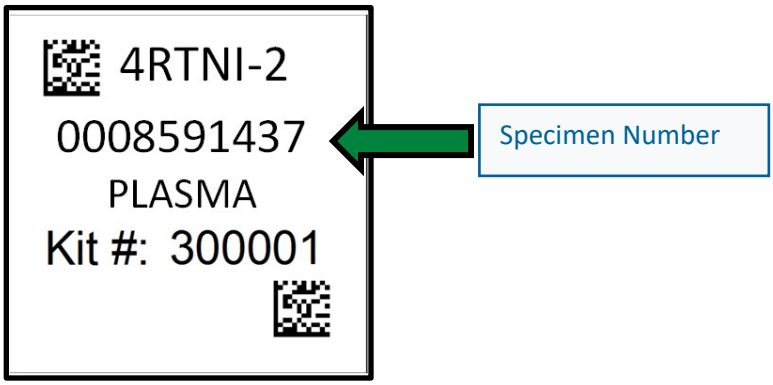
Serum Determination Tube

PAXgene™ Tube

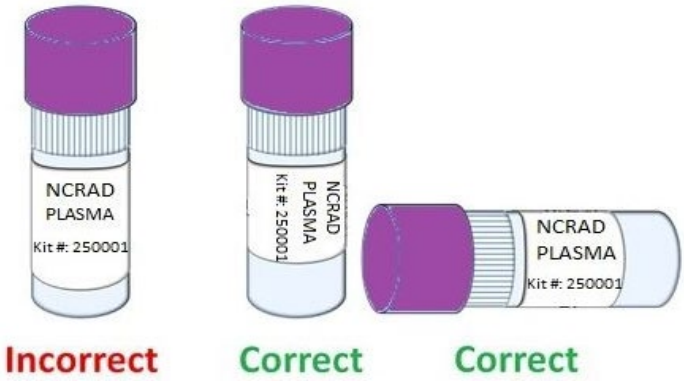
In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place blood Collection and Aliquot Tube Labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing, or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.

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



- Using a fine point sharpie, fill-in and place the RAVE ID labels on the collection tubes only (EDTA, Sodium Heparin, Serum, and PAXgene™) **BEFORE** sample collection, sample processing or freezing. These labels are in addition to the Kit Number Labels. **DO NOT** place RAVE ID labels on any cryovials.
- The blood Collection and Aliquot Tube Labels contain a 2D barcode on the left 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.



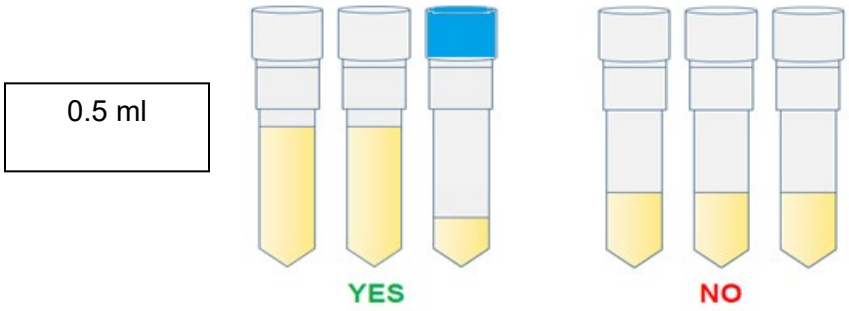
6.2 Video List

- The following training videos are available to assist you with the specimen processing, aliquoting, and shipping processes. The videos are available at https://ncrad.org/resource_4rtni.html.

  - Frozen Shipping
  - Ambient Shipping
  - Plasma and Buffy Coat Processing and Aliquoting
  - Serum Processing and Aliquoting
  - RNA (PAXgene™ Tube) Processing
  - CSF Processing and Aliquoting
  - 4RTNI-2 MOP Training

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

In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to the assigned volume after processing is completed (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of sample. Aliquot the remaining biologic material as the residual volume and ship to NCRAD. Ship *all* material to NCRAD. Fill as many aliquot tubes as possible. For example, if 2.7 ml of a plasma sample is obtained, fill 5 cryovial tubes each with 0.5 ml, and one additional cryovial tube with the remaining 0.2 ml.



Please note: It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume (anything under 0.5 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 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
Clear	CSF Aliquot (0.5 ml)
Orange	CSF Aliquot (1.0 ml)
Orange	CSF Aliquot to local lab
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: EDTA (Lavender-Top) Blood Collection Tubes (10 ml) (for processing of plasma aliquots and buffy coat aliquots).

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

At the baseline visit, draw THREE:

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

At longitudinal visits, draw TWO:

- **EDTA (Lavender-Top) Blood Collection tube (10 ml) for Buffy Coat and Plasma**

1. 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 2 ml cryovial tubes with lavender caps. Place pre-printed "**BUFFY COAT**" aliquot label on the 2 ml cryovial with a clear cap.

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

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

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

The following techniques shall be used to prevent possible backflow:

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

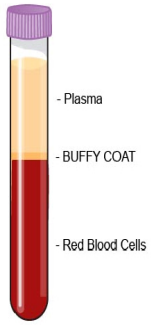
d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

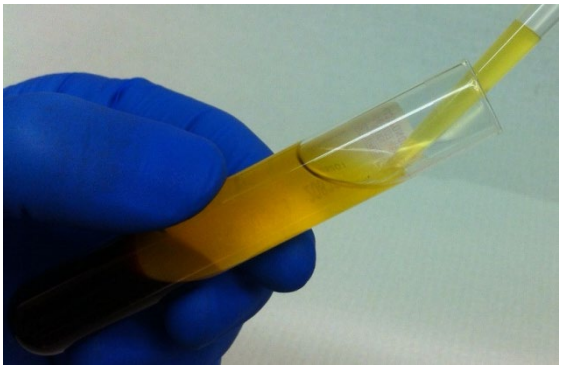
5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into each tube.

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

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

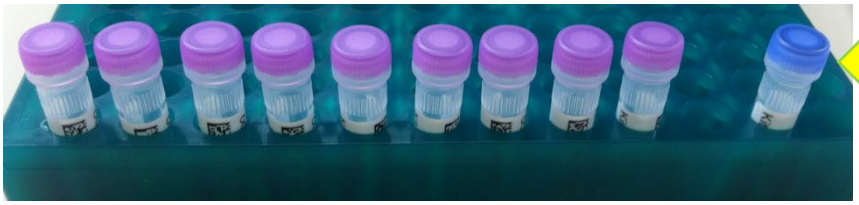
8. Remove the plasma, being careful not to agitate the packed blood cells at the bottom of the collection tube, by tilting the tube and placing the pipette tip along the lower side of the wall without touching the pellet so that plasma is not contaminated by pellet material (see below). Using a disposable graduated transfer pipette, transfer plasma into the pre-labeled cryovials. Aliquot 0.5 ml per cryovial (with 0.5 ml each). Each EDTA tube should yield, on average, 5 ml of blood plasma per tube. Be sure to only place **plasma** in cryovials labeled with **"PLASMA"** labels**.** Take caution not to disturb the blood cells (cell pellet) at the bottom of the tube. If there is extra plasma left, use 1 extra cryovial provided in the supplemental kit for another 0.5ml aliquot of plasma. **If a residual aliquot (<0.5 ml) is created, utilize the cryovial with the blue cap to highlight which aliquot contains a smaller volume. Document the sample number 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 (24-30 total possible at baseline; 16-20 total possible at longitudinal visits

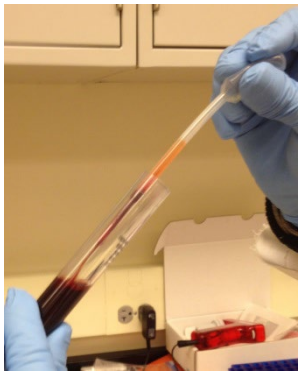


Residual Aliquot with Blue Cap

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

10. After plasma has been removed from each EDTA (Lavender-Top) Blood Collection Tube (10 ml), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) into the labeled cryovials with clear cap using a clean disposable graduated transfer pipette. Buffy coat from each EDTA tube will be placed in a separate 2.0 ml cryovial with clear cap, so a total of two to 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 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)



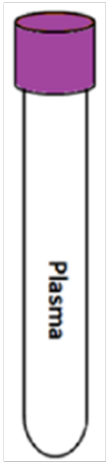
Buffy Coat Aliquot

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

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

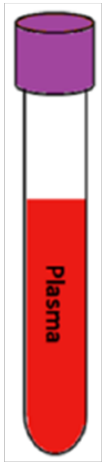
Plasma and Buffy Coat Preparation (10ml Purple Top Tube)

Step One



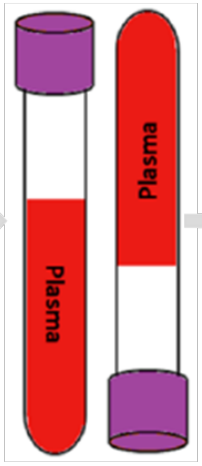
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



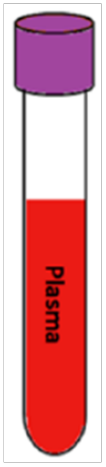
- Collect blood in EDTA Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



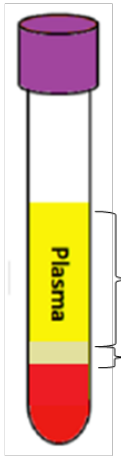
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

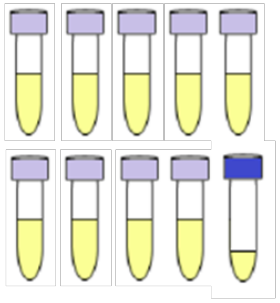
Step Four



- Centrifuge samples at 1500 x g for 15 minutes at 4°C.
- EDTA tubes need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.

Step Five





- Label cryovial tubes with preprinted labels.
- Aliquot 0.5 ml into each cryovial tube.
- If residual aliquot is created, use blue cap to indicate volume difference and document Specimen Number on Biological Sample and Shipment Notification Form.
- Store plasma aliquots at -80°C until shipment.

Step Six

- Label cryovial tube with preprinted label.
- Using a clean transfer pipette, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat into the cryovial tube.
- Store buffy coat aliquot at -80°C until shipment.

6.5 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 the Baseline study visit.

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

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

Therefore, this blood tube CANNOT be drawn on a Friday.

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

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

The following techniques shall be used to prevent possible backflow:

   a. Place 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.

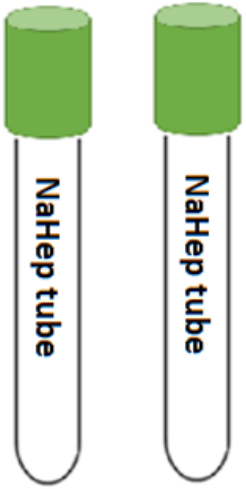
3. Allow at least 10 seconds for a complete blood draw to take place in the tube. **Ensure that the blood has stopped flowing into each tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.

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

5. Ship both unprocessed tubes *ambient* to NCRAD the day of the participant visit. Please see Section 8.2 for detailed ambient shipping instructions.

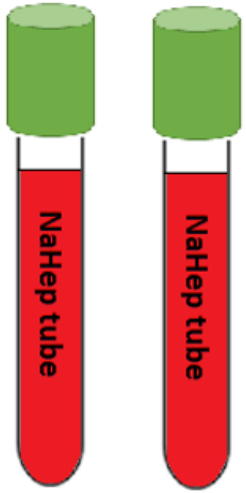
PBMC Preparation (10ml Sodium Heparin Tube x 2)

Step One



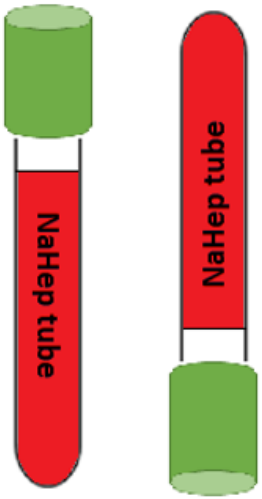
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



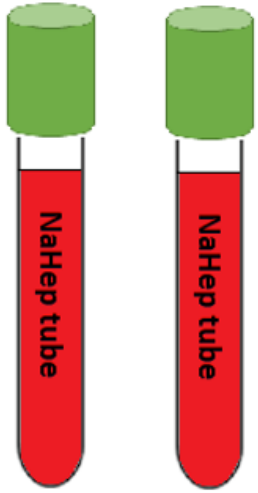
- Collect blood in Sodium Heparin Tubes allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four



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

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 the Baseline study visit.

1. Place completed Site and RAVE ID and pre-printed "SERUM" collection tube label on the red-top serum tube. Place pre-printed "**SERUM**" labels on the (10) 2 ml cryovial tubes with red caps.

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

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

4. 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.

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

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

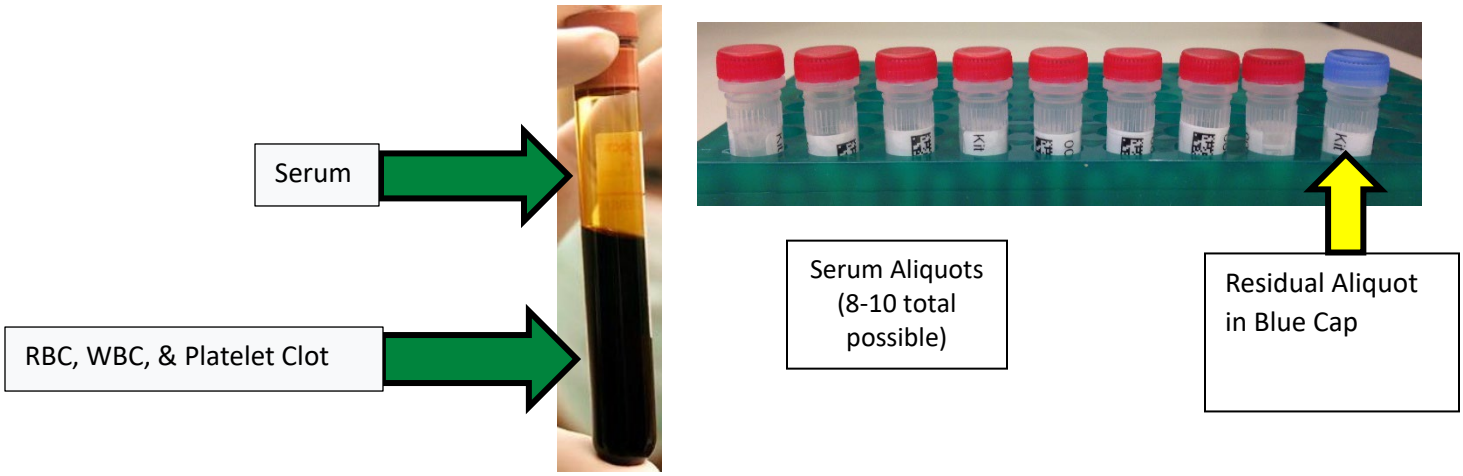
7. **CRITICAL STEP: Allow blood to clot at room temperature by placing it upright in a vertical position in a tube rack for 30 minutes.**

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

9. Remove the serum, being careful not to disturb the clot at the bottom of the collection tube by tilting the tube and placing the 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 0.5 ml per cryovial (total vials=8-10 with 0.5 ml each). The red-top tube should yield, on average, 5 ml of blood serum for a total of 8-10 2 ml aliquot cryovial tubes per participant with 0.5 ml per cryovial tube. Be sure to only place **serum** in cryovials with red lids labeled with the **"SERUM"** label. If there is extra serum left, use 1 extra cryovial provided in the supplemental kit for another 0.5ml aliquot of plasma. **If a residual aliquot (<0.5 ml) is created, utilize the cryovial with the blue cap to highlight which aliquot contains a smaller volume. Document the sample number on the Biological Sample and Shipment Notification Form.**

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

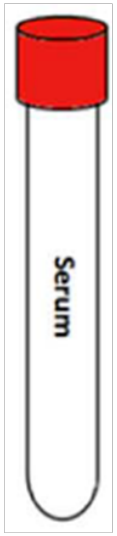
10. Place the labeled cryovials in the 81-slot cryobox and place on dry ice pellets. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice pellets.



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

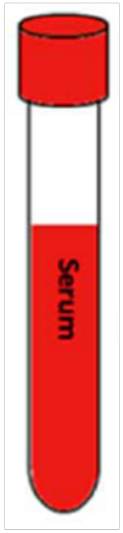
Serum Preparation (10ml Red Top Tube)

Step One



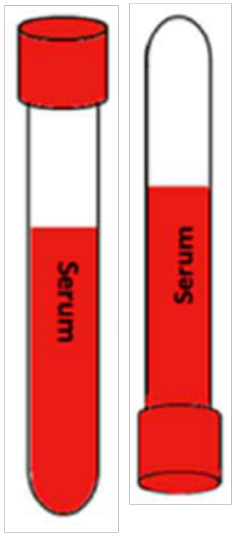
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



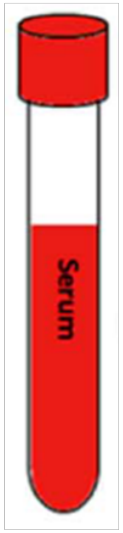
- Collect blood in Serum Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



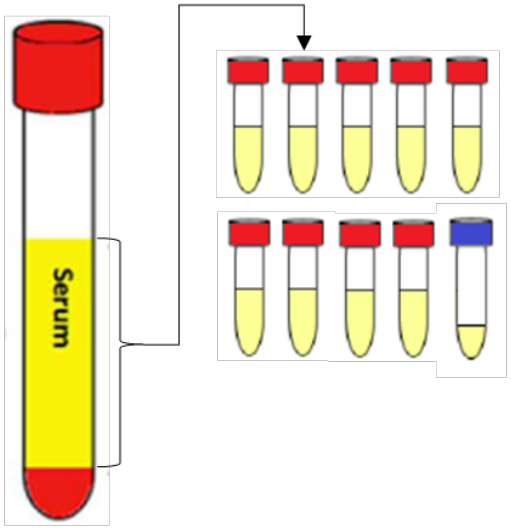
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four



- Allow blood to clot for 30 minutes.
- Centrifuge samples at 1500 x g for 15 minutes at 4°C.
- Serum samples need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.

Step Five



- Label cryovial tubes with preprinted labels.
- Aliquot 0.5 ml into each cryovial tube.
- If residual aliquot is created, use blue cap to indicate volume difference and document Specimen Number on Biological Sample and Shipment Notification Form.
- Store plasma aliquots at -80°C until shipment.

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

See training videos for blood collection: (http://www.preanalytix.com/videos/rna-tube-collection-video/)

Whole Blood Collection for Isolation of RNA: three PAXgene™ Blood Collection Tubes for RNA. Three PAXgene™ tubes are collected at the Baseline study visit.

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

Draw the PAXgene™ tubes LAST, after all other specimens are collected for the 4RTNI-2 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.

1. **CRITICAL STEP: 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 three 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 **three 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.
   e. PAXgene™ tubes should be collected LAST. Please refer to Section 6.0 for the order of the blood draw.

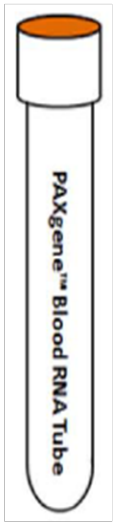
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The PAXgene™ Blood RNA Tube with its vacuum is designed to draw 2.5ml of blood into the tube. Record total amount of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

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

5. Place the PAXgene™ tubes upright in a **WIRE** or **PLASTIC** rack. Transfer to **-80°C Freezer when possible**. 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.

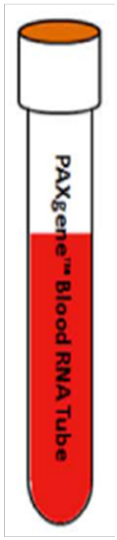
RNA Preparation (2.5ml PAXgene™ Tube)

Step One



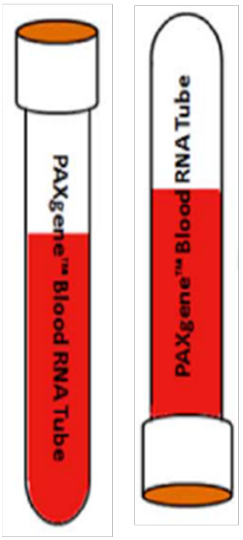
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



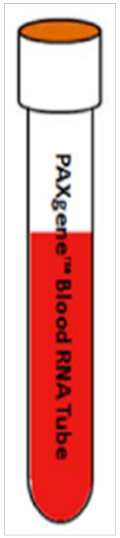
- Collect blood in PAXgene™ tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four



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

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 that failed and must 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 for a redraw, is going to be used for DNA only, and is not going to be spun down for plasma isolation, 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 4RTNI-2 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 F and Appendix G for Biological Shipping Forms for participants who are provided blood kits for their local physicians.

7.0 Cerebrospinal Fluid Collection

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

CSF should be collected in the morning between 8am – 10am, preferably fasted. If fasting is not feasible, the low fat diet should be followed (See Appendix E). 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 Indiana University.

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 who are 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 5.2).

7.2 Setting Up the LP

1. On an over bed table, remove the contents of the LP kit from the outer plastic packaging, leaving the contents wrapped in their sterile drape. 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_4rtni.html .

Place the "**CSF**" label on the collection and aliquot tubes (per Section 6.1). Please remember, the kit number on the CSF labels will be different than the blood aliquots. Prepare the 34 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 4RTNI-2 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.*

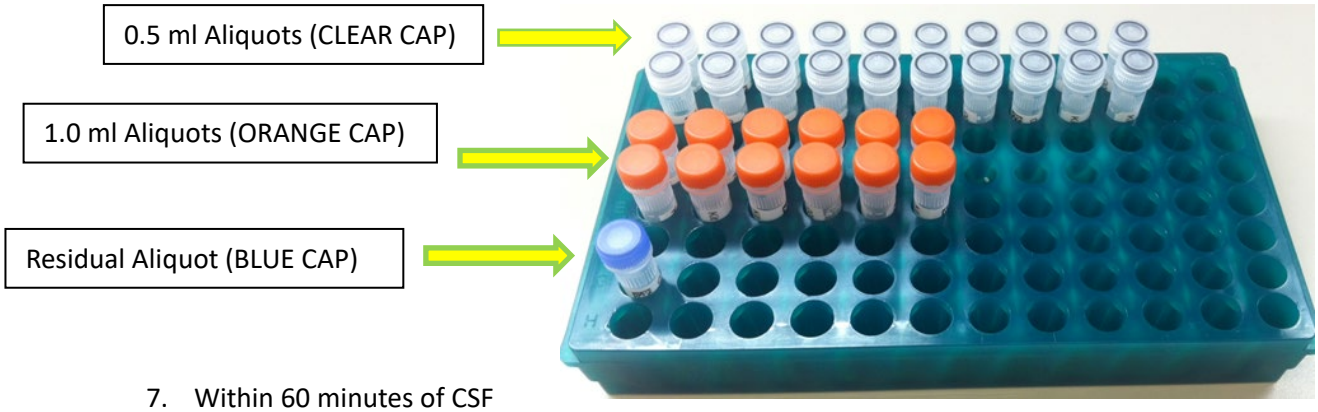
4. Collect an additional 23 mls of CSF and transfer to 50 ml conical polypropylene tubes at room temperature. Mix gently by inverting 3-4 times. Record the time of draw (once collection is complete) on the CSF Sample and Shipment Notification Form.

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) at least 0.5 ml of supernatant directly into pre-cooled polypropylene CSF collection aliquot tubes. The first 10 mls of the supernatant will be separated into 0.5 ml aliquots (utilize CLEAR cap cryovials here). All remaining supernatant will be separated into 1.0 ml aliquots (utilize ORANGE cap cryovials here). This will yield, on average, 33 aliquot tubes per participant. (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 (<0.5 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.



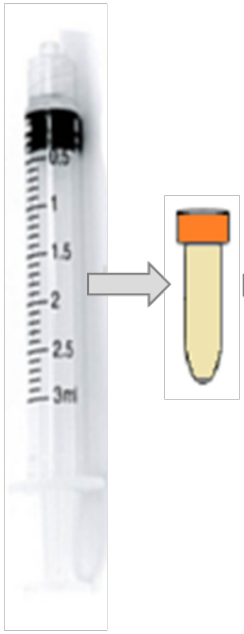
7. Within 60 minutes of CSF collection, freeze aliquots immediately on dry ice and then store at **-80°C** or ship on dry ice in a shipping container. Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the RAVE database.

CSF Preparation (20-25 mL in Syringes)

Step One

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

Step Two

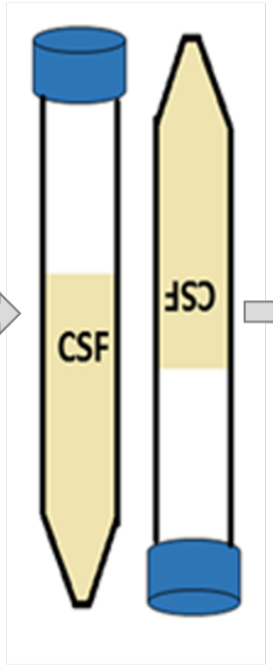


- Collect CSF into the 3mL luer lock syringe.
- Dispense 1-2mL in ORANGE cap cryovial.
- Send to local lab for testing.

Step Three

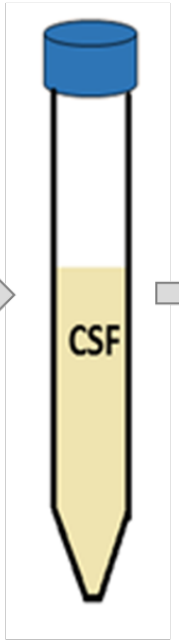
- Collect CSF into 6mL luer lock syringe.
- Collect 20-23 mL.

Step Four



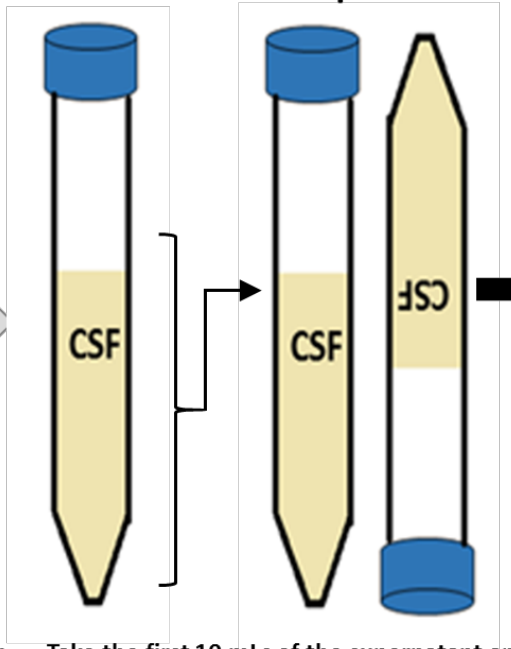
- Immediately after collection, transfer to 50 mL conical tube.
- Invert tube 3-4 times to mix sample.

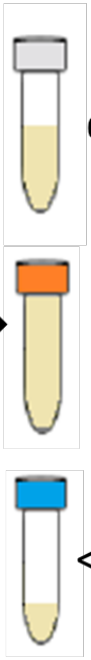
Step Five



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

Step Six





- Take the first 10 mLs of the supernatant and separate into 0.5 mL aliquots. Use CLEAR CAPS for this step.
- Aliquot the remaining supernatant into 1.0 mL aliquots. Use the ORANGE CAPS for this step.
- Aliquot any remaining residuals into the BLUE CAP cryovials.
- Document specimen number of residual aliquot on sample form
- Store CSF aliquots at -80° until shipment
- 1-3 mL may stay at local lab for researcher, if 20 mL of CSF is submitted to NCRAD

8.0 Packaging & Shipping Instructions

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

Sample Type	4RTNI-2 Study Visit	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood (Lavender-Top EDTA) for isolation of plasma & buffy coat (for DNA extraction)	**All Cycles**	0.5 ml plasma aliquots per 2 ml cryovials	24-31 (Cycle 1) 16-21 (Cycle 2-4)	Frozen
	All Cycles	1 ml buffy coat aliquot per 2 ml cryovial	3	Frozen
Whole blood (Green-Top Sodium Heparin) for isolation of PBMCs	**Cycle 1**	N/A	2	Ambient
Whole blood (Red-Top Serum) for isolation of serum	**Cycle 1**	0.5 ml serum aliquots per 2 ml cryovials	8-11	Frozen
Whole blood (PAXgene™) for RNA extraction	**Cycle 1**	N/A	3	Frozen
CSF	**Cycle 1, 3, and 4 (Visit 2 if Termination Visit)**	0.5 ml and 1 ml CSF aliquots per 2 ml cryovials	Up to 33	Frozen

8.1 Frozen Shipping

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.

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 (PAXgene™ RNA tubes or frozen cryovials) must be leak proof and must not contain more than 1L total.
- The secondary packaging (bubble-wrap or biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (within the cryovial box containing the frozen cryovials or PAXgene™ RNA tubes) 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
  - ✓ Class 9 label including UN 1845, and net weight of dry ice contained

conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

8.1.1 NCRAD Packaging and Shipment Instructions – Frozen Shipments

1. 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. Contact FedEx to confirm service is available and schedule package to be picked up.

3. 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.

4. Place all frozen labeled 0.5 ml aliquots of plasma, buffy coat, serum, and CSF in the one 81-slot cryobox.

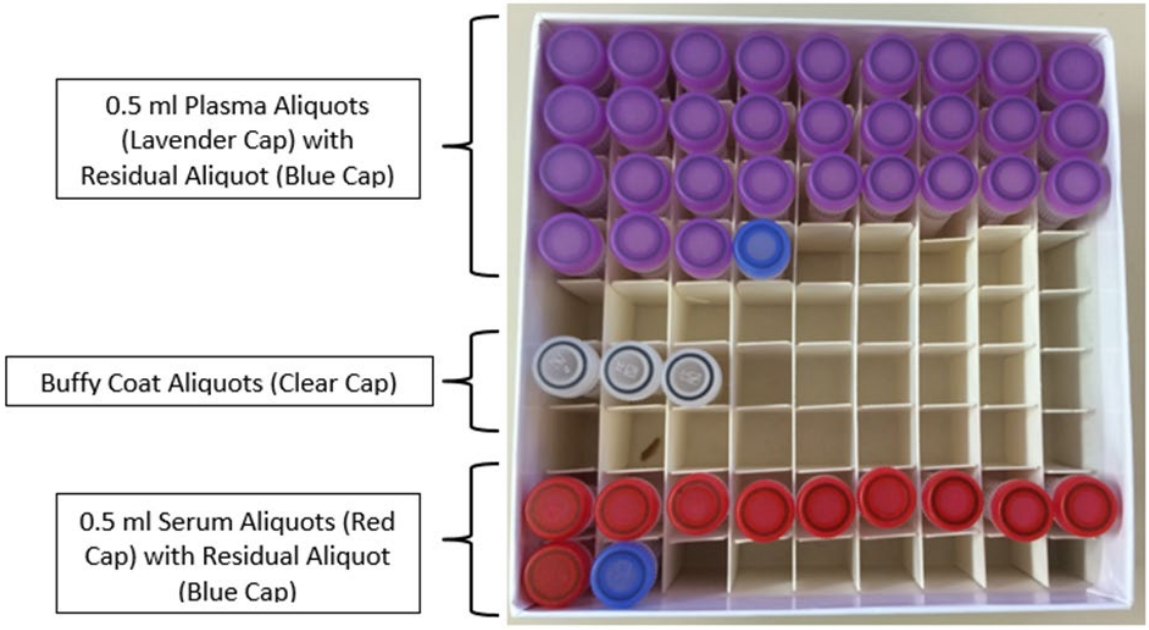
    i. Each cryobox holds up to 81 cryovials and there will be a maximum of 45 blood-derived aliquots (31 plasma, 11 serum, and 3 buffy coat) per Baseline blood draw (see next page).
        1. Longitudinal visits will contain a maximum of 23 blood-derived aliquots (21 plasma, 2 buffy coat).

    ii. If CSF draw is drawn at that time point, include the CSF aliquots with the blood-derived aliquots (33 cryovials). (see next page)

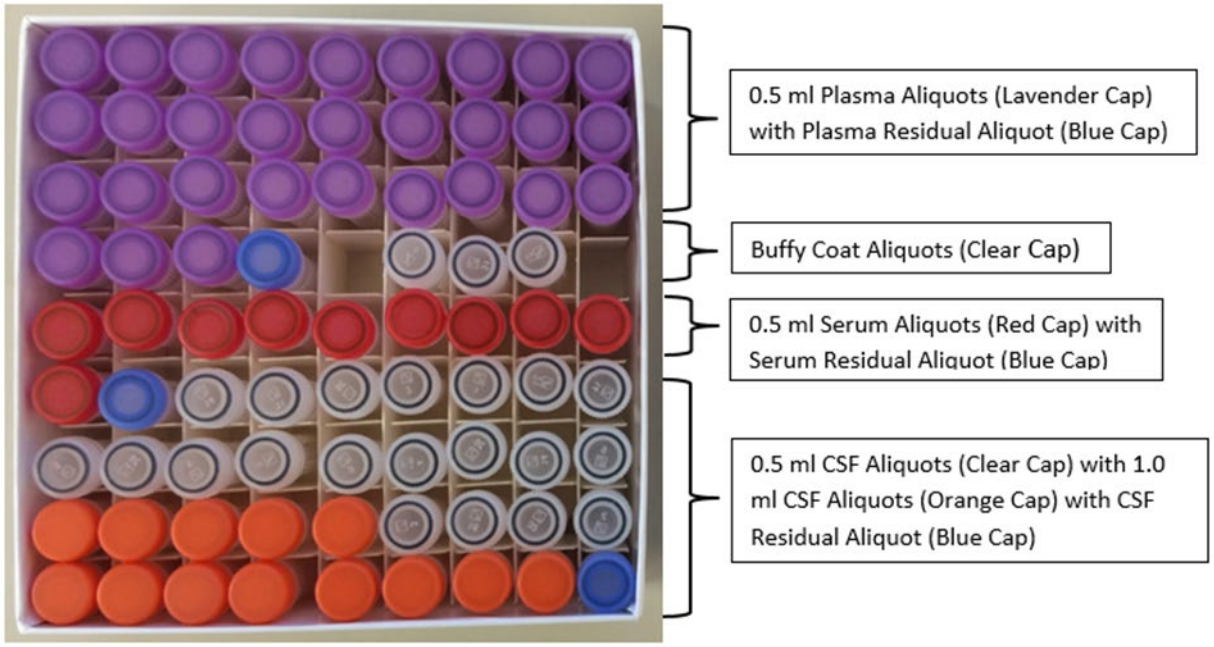
    iii. A cryobox should contain all of the specimens from the same patient, per time point.

    iv. **Batch shipping should be performed quarterly or when specimens from 4 participants accumulates, whichever is sooner.**

81-Slot Cryobox Containing Biospecimens from One Baseline Blood Visit



81-Slot Cryobox Containing Biospecimens from One Baseline Blood Visit with CSF Collected



5. Label the outside of each cryobox with the kit number label(s). Please place the cryoboxes containing blood derivatives (and CSF, if drawn) in one biohazard bag.

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

7. As the cryoboxes and PAXgene™ tube are placed in the large 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(s) should be placed on each cardboard cryobox prior to inserting into the biohazard bag. A kit number label should also be placed on the outside of the biohazard bag.

Cryobox and PAXgene™ placed in clear biohazard bag

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

9. Place the biohazard bag into the provided Styrofoam-lined shipping container on top of the dry ice pellets. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container. **A maximum of 4 cryoboxes may be sent in each shipper.**

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

11. The inner Styrofoam shipping container must contain approximately 45 lbs (or 20kg) of dry ice pellets. 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.

12. 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.

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

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

4RTNI-2 at NCRAD
Indiana University School of Medicine
351 W. 10th St. TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

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

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

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

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

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

4RTNI-2 at NCRAD
Indiana University School of Medicine
351 W. 10th St. TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

18. Use FedEx 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 FedEx tracking number in your email.

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

For frozen shipments, include no more than four cryovial boxes (separated by patient within biohazard bags) and four bubble-wrap sleeves 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 will be shipped to NCRAD as outlined above.

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

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

1.1

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 Shipping 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 canister. The ambient PBMC samples must be shipped the day of blood draw.

Therefore, this blood tube CANNOT be drawn on a Friday.

The labeled, unprocessed, sodium heparin PBMC tubes 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 (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.

***** 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 (plastic canister) must be leak proof.
- Absorbent material must be placed between the primary receptacle (sodium heparin tube) and the secondary packaging (plastic canister). 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

8.2.1 NCRAD Packaging and Shipment Instructions (Ambient Shipments)

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

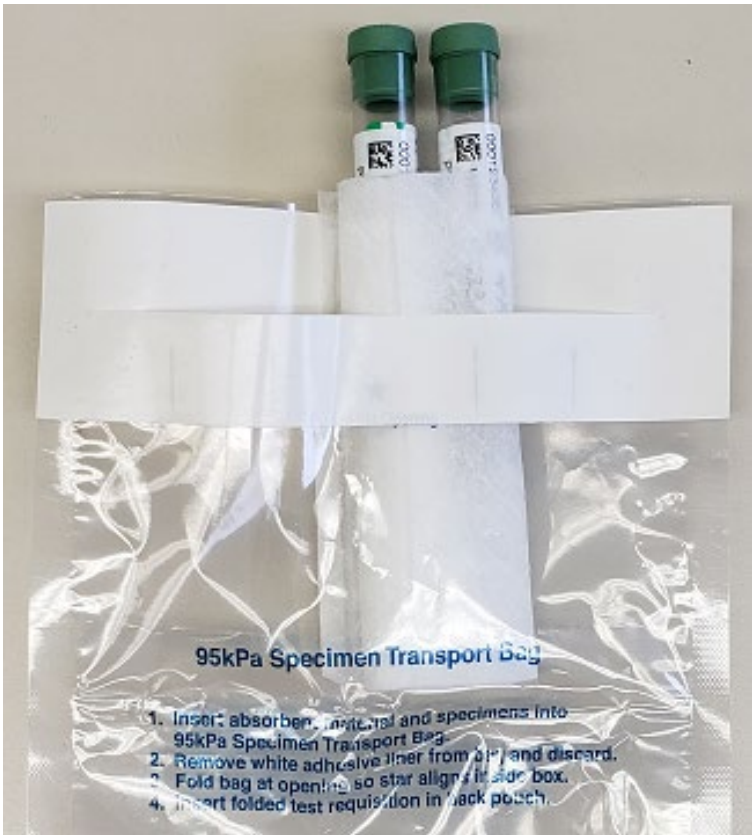
2. Contact FedEx to confirm service is available and schedule package to be picked up.

3. 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)

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

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

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



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

8. Place the lid onto the cooler.

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

10. Close shipping box and place within a Fed-Ex Clinical Pak. **Seal the Fed-Ex Clinical Pak.**

11. Place prefilled FedEx return airbill to the sealed Fed-Ex Clinical Pak.
    a. Be sure to complete the FedEx return airbill with the following information:
        - Section 1, "From": fill in the date, your name, phone number, and Site FedEx Account Number.
        - Section 2, "Your Internal Billing Reference": add any additional information required by your site.

12. Specimens should be sent to the below address via FedEx Priority Overnight. Ambient FedEx shipments should be sent Monday through Thursday.

4RTNI-2 at NCRAD
Indiana University School of Medicine
351 W. 10th St. TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

13. Use FedEx tracking to ensure the delivery occurs as scheduled and is received by NCRAD.

8.3 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 Shipping Instructions) and Section 8.2 (Ambient Shipping Instructions).

2. Two components are necessary for international shipments:
    1. International FedEx return airbill
    2. International Commercial Invoice

3. NCRAD will provide an International FedEx return airbill to all Canadian Sites.
    a. Be sure to complete the FedEx return airbill with the following information:

        i. Section 1, From: Enter the date and your name, phone number, complete address, and FedEx account number.

        ii. Section 2, To: This information will be preprinted with NCRAD's return address and phone number.

        iii. Section 3, Shipment information: This information does NOT replace a Commercial Invoice that is required for these shipments. Total Packages, Weight, and box dimensions are required. Be consistent between this International FedEx return airbill and the International Commercial Invoice.
            1. Do not declare the value of the shipment to be over $2,500. This would require additional paperwork (a Shipper's Export Declaration form).

        iv. Section 4, Express Package Services: Please check FedEx Intl. Priority for both Frozen and Ambient Shipments. (Pictured)

        v. Section 5, Packaging: Please select "Other" for Frozen Shipments and "FedEx Pak" for Ambient Shipments.

        vi. Section 6, Special Handling: Please leave blank.

        vii. Section 7 and 8, Payment: Check Sender and bill transportation costs to your site study FedEx account number. Duties and Taxes will also be billed to the sender. If your site requests information to be included as reference, please complete Section 8.

        viii. Section 9, Required Signature: This section must be signed by the sender or department representative.

    b. International Commercial Invoice (See Appendix D)

i. The International Commercial Invoice must be completed and placed with the International return airbill.

   1. Include **ONE** original and **THREE** copies of this completed form with the FedEx return airbill.

ii. Complete "Shipped From" with your name, address, and any additional contact information.

iii. Complete "Shipped To, Consignee" with the NCRAD shipping address:

4RTNI-2 at NCRAD
Indiana University School of Medicine
351 W. 10th St. TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

iv. Complete Number of Packages and Shipping weight to match the information recorded within the International FedEx return airbill.

v. Immediately below the shipping weight is a section asking for the Country of Origin, Description of Goods, Quantity, Unit Price, and Total Price. Please be as detailed as possible within this section (example pictured below).

COUNTRY OF ORIGIN & PROVINCE, IF CANADA *PAYS D'ORIGINE ET PROVINCE, SI CANADA*	DESCRIPTION OF GOODS *DESCRIPTION DES MARCHANDISES*	QUANTITY *QUANTITÉ*	UNIT PRICE *PRIX UNITAIRE*	TOTAL PRICE *PRIX TOTAL*
Canada, Vancouver	Non-Infectious, non-contagious, human Plasma and Buffy Coat sample	1 Box (11 Aliquots)	100.00	100.00

vi. Tally the Total Price within the last column for all goods included in shipment and record appropriately.

   1. Reminder: the total price/value of the shipment should not exceed $2,500.

vii. Complete the final section with your signature.

viii. Specimens should be sent to the below address via FedEx Priority Overnight. Ambient FedEx shipments should be sent Monday through Thursday. Frozen FedEx Shipments should only be sent Monday through Wednesday.

ix. Use FedEx tracking to ensure the delivery occurs as scheduled and is received by NCRAD.

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 iMedidata RAVE data collection team will be collaborating with NCRAD to reconcile information captured in the database compared to samples received and logged at NCRAD. Information that appears incorrect in the 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 4RTNI-2 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

317-278-2003 (Fax) **alzstudy@iu.edu**

Appendix B

Biological Sample and Shipment Notification Form

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

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

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

From: _____ Site: _____

Phone: _____ Fax: _____

Email: _____ Date: _____

Study: 4RTNI-2 **Kit #:**

Visit: _____

KIT BARCODE

Site ID: _____ **RAVE #:** _____

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

Blood Collection:

1. Date Drawn: _____ [YYYYMMDD]	2. Time of Draw: 24 hour clock: _____ [HHMM]
3. Last time participant ate: Date: _____ [YYYYMMDD]	4. Last time participant ate: Time: 24 hour clock: _____ [HHMM]
5. **Baseline Only: Sodium heparin tubes (PBMC) drawn 2 x 10mL:** ☐ Yes ☐ No	
6. **Baseline Only: Total volume of blood drawn into 3 x 2.5 ml PAXgene™ RNA tubes:** _____ mL • Were the PAXgene™ tubes the last tubes drawn? ☐ Yes ☐ No	

Blood Processing:

Plasma (EDTA Tube)		**Serum (Serum Determination Tube) Baseline Only**	
Time spin started: 24 hour clock:	_____ [HHMM]	Time spin started: 24 hour clock: (within 30 minutes of draw time):	_____ [HHMM]
Original volume drawn (2 x 10 mL or 3 x 10 mL EDTA tube):	_____ mL	Original volume drawn (1x10 mL Serum tube):	_____ mL
Number of 0.5 mL plasma aliquots created (24-30 BL) or (14-20 LONG): **(Lavender cap cryovial):**	_____ x 0.5 mL	Number of 0.5 mL serum aliquots created (8-10 total): **(Red cap cryovial):**	_____ x 0.5 mL
If applicable, volume of residual plasma aliquot (less than 0.5 mL): **(Blue cap cryovial):**	_____ mL	If applicable, volume of residual serum aliquot (less than 0.5 mL): **(Blue cap cryovial):**	_____ mL
If applicable, specimen number of residual aliquot: **(Last four digits)**	_____	If applicable, specimen number of residual aliquot: **(Last four digits)**	_____
Buffy coat aliquots created (one per EDTA tube): **(Clear cap cryovial)**:	_____		
Time aliquots placed in freezer: 24 hour clock:	_____ [HHMM]	Time aliquots placed in freezer: 24 hour clock:	_____ [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: 317-321-2003 Phone: 1-800-526-2839

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

From: _____ Site: _____

Phone: _____ Fax: _____

Email: _____ Date: _____

Study: 4RTNI-2 **Kit #:**

KIT BARCODE

Site ID: _____ **RAVE #:** _____

Fam #: _____ **Sex:** ☐ M ☐ F **Year of Birth:** _____ **Visit:** _____

CSF Collection:

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

CSF Processing:

1. Total number of CSF **aliquot** tubes: _____	4. Total number of CSF **0.5 mL aliquots transferred** to NCRAD: **(Clear cap cryovial)**: _____
2. Total amount of CSF **collected** (mL): _____	5. Total number of CSF **1.0 mL aliquots transferred** to NCRAD: **(Orange cap cryovial)**: _____
3. Time frozen: _____ [HHMM]	6. If applicable, volume of CSF residual aliquot (less than 0.5 mL): **(Blue cap cryovial)**: _____
7. If applicable, specimen number of residual aliquot tube: **(Last four digits)**: _____	

Notes: _____

Appendix D
International Customs Declaration Worksheet

FOR CUSTOMS CLEARANCE BY / POUR DÉDOUANEMENT PAR

FedEx Trade Networks

INVOICE
FACTURE

STAPLE LINE RELEASE LABEL HERE

EXPORTER, SELLER, CONSIGNOR *EXPORTATEUR, VENDEUR, EXPÉDITEUR* / TELEPHONE *TÉLÉPHONE*	SHIPPED FROM (IF DIFFERENT THAN EXPORTER) *EXPÉDIÉ (SI DIFFÉRENT DE L'EXPORTATEUR)*
U.S. IRS NUMBER OR S/S NUMBER / REFERENCE *RÉFÉRENCE*	
BUYER (IF SOLD) *ACHETEUR (SI VENDU)* / TELEPHONE *TÉLÉPHONE*	SHIPPED TO, CONSIGNEE (IF NOT SOLD OR IF DIFFERENT THAN BUYER) *EXPÉDIÉ AU DESTINATAIRE (SI NON VENDU OU SI AUTRE QUE L'ACHETEUR)*
U.S. IRS NUMBER OR S/S NUMBER / REFERENCE *RÉFÉRENCE*	U.S. IRS NUMBER OR S/S NUMBER / REFERENCE *RÉFÉRENCE*
PARTIES TO THIS TRANSACTION ARE *LES TRANSACTIONNAIRES SONT* ☐ RELATED *ASSOCIÉS* ☐ NOT RELATED *NON ASSOCIÉS* / COUNTRY OF FINAL DEST. (IF OTHER THAN U.S.A.) *PAYS DE DESTINATION FINALE (SI AUTRE QUE LES É.-U.)*	INVOICE NUMBER *NUMÉRO DE FACTURE*
U.S. DUTY AND/OR BROKERAGE FOR / *DROITS DE DOUANE AMÉRICAINE ET/OU FRAIS DE COURTAGE POUR* ☐ EXPORTER (INCLUDED) *(COMPRIS)* ☐ EXPORTER (NOT INCLUDED) *(NON COMPRIS)* ☐ BUYER *ACHETEUR* ☐ CONSIGNEE *DESTINATAIRE*	INVOICE DATE *DATE DE LA FACTURE* / IMPORTING CARRIER/*TRANSPORTEUR À L'IMPORTATION*
OR FOR THE ACCOUNT OF *OU POUR LE COMPTE DE*	DATE OF SALE *DATE DE LA VENTE*
	DISCOUNTS *ESCOMPTES*
	CURRENCY OF VALUE *VALEUR EN DEVISES* ☐ US ☐ CANADIAN *CANADIENNES* OTHER *AUTRE* ☐

MARKS AND NUMBERS *MARQUES ET NUMÉROS*	NUMBER AND KIND OF PACKAGES *NOMBRE ET CATÉGORIE DE COLIS*	SHIPPING WEIGHT *POIDS DE L'EXPÉDITION*	FREIGHT AMOUNT INCLUDED *MONTANT DU FRET COMPRIS*	FREIGHT AMOUNT TO BORDER *MONTANT DU FRET À LA FRONTIÈRE*

COUNTRY OF ORIGIN & PROVINCE, IF CANADA *PAYS D'ORIGINE ET PROVINCE, SI CANADA*	DESCRIPTION OF GOODS *DESCRIPTION DES MARCHANDISES*	QUANTITY *QUANTITÉ*	UNIT PRICE *PRIX UNITAIRE*	TOTAL PRICE *PRIX TOTAL*

Cargo Insurance is available through FedEx Trade Networks. Please contact Customer Service at 1.800.249.2953 for a competitive quote.
L'assurance cargo est offerte par l'entremise de FedEx Trade Networks. Veuillez contacter le service à la clientèle au 1.800.249.2953 pour une estimation compétitive.

INVOICE TOTAL
TOTAL DE LA FACTURE

DECLARATION BY FOREIGN SHIPPER (COMPLETE IF GOODS ARE OF U.S. ORIGIN AND VALUE EXCEEDS $2000.00)

I, _____, DECLARE THAT TO THE BEST OF MY KNOWLEDGE AND BELIEF THE ARTICLES HEREIN SPECIFIED WERE EXPORTED FROM THE UNITED STATES, FROM THE PORT OF _____ ON OR ABOUT _____ AND THAT THEY ARE RETURNED WITHOUT HAVING BEEN ADVANCED IN VALUE OR IMPROVED IN CONDITION BY ANY PROCESS OF MANUFACTURE OR OTHER MEANS.

SIGNATURE _____ CAPACITY _____

To the best of the knowledge and belief of the preparer this invoice is true and complete and discloses the true prices, values, quantities, rebates, drawbacks, fees, commissions, royalties and any goods or services provided to the seller either free or at a reduced cost.	PREPARER (IF OTHER THAN EXPORTER) *PRÉPARATEUR (SI AUTRE QUE L'EXPORTATEUR)*	NAME OF RESPONSIBLE EMPLOYEE OF EXPORTER *NOM DE L'EMPLOYÉ RESPONSABLE CHEZ L'EXPORTATEUR*

FORM 12 3/05 WV

For a list of FedEx Trade Networks office locations call 1.800.249.2953

Appendix E
Low Fat Diet Menu Suggestions

Foods to avoid prior to blood collection:

Avoid: *All Fats and nuts such as:*

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

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

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

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

All red meats or meats containing fat such as pork

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

Appendix F
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 FedEx Clinical Pak.

4RTNI-2 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 **Federal Express** immediately after drawing. Use the enclosed, pre-paid Federal Express mailer. There will be no cost to you or the patient for the shipping.

☐ 4RTNI-2 ☐ ARTFL

KIT NUMBER (RECORDED ON LABEL): _____

RAVE IDENTIFICATION NUMBER (RECORDED ON LABEL): _____

STUDY SITE ID (RECORDED ON LABEL): _____

DATE BLOOD WAS DRAWN: _____

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

Appendix G
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 FedEx Clinical Pak.

4RTNI-2 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 **Federal Express** immediately after drawing. Use the enclosed, pre-paid Federal Express mailer. There will be no cost to you or the patient for the shipping.

☐ 4RTNI-2 ☐ ARTFL

KIT NUMBER (RECORDED ON LABEL): _____

RAVE IDENTIFICATION NUMBER (RECORDED ON LABEL): _____

STUDY SITE ID (RECORDED ON LABEL): _____

DATE BLOOD WAS DRAWN: _____

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