



Manual of Procedures

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

T2 Protect AD Open Label Extension

Biospecimen Collection, Processing, and Shipment Manual

Version 12.2020

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1.0 Abbreviations

AD	Alzheimer's Disease
ADCS	Alzheimer's Disease Cooperative Study
BL	Baseline Visit
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
GUID	Globally Unique Identifier
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PHI	Protective Health Information
RBCs	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

2.0 Purpose

The purpose of this manual is to provide T2 PROTECT AD staff (PIs, study coordinators, and the sample collection and processing teams) at the various study sites with instructions for collection and submission of biological samples for T2 PROTECT AD 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:

- Serum
- Plasma
- Buffy Coat (DNA Extraction)

This manual includes instructions for collection and 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 T2 PROTECT AD protocols.

3.0 NCRAD Information

3.1 NCRAD Contacts

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Phone: 317-274-7360

Email: kelfaber@iu.edu

Milena Petkov, BS, CCRP, Study Coordinator

Phone: 317-278-1228

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General NCRAD Contact Information

Phone: 1-800-526-2839

Fax: 317-321-2003

Email: alzstudy@iu.edu

Website: www.ncrad.org

T2 PROTECT AD Study Specific Webpage: https://ncrad.org/resource_t2.html

Sample Shipment Mailing Address

NCRAD

Indiana University School of Medicine

351 W. 10th St

TK-217

Indianapolis, IN 46202

3.2 Hours of Operation

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

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

Samples should be shipped to NCRAD within 3 months of the sample collection date to mitigate sample expiration concerns. Check weather report to make sure

impending weather events (blizzards, hurricanes, etc.) will not affect the shipping or delivery of the samples.

3.3 Holiday Schedules

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

3.4 Holiday Observations

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

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

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

4.0 Globally Unique Identifier (GUID)

The GUID is a subject ID that allows researchers to share data specific to a study participant, without exposing personally identifiable information. A GUID is made up of

random alpha-numeric characters and does not include any PHI in the identifier. By using GUIDs in your research data, the system can associate a single research participant's genetic, imaging, and clinical assessment data even if the data was collected at different locations or throughout different studies.

To create a GUID follow these steps:

1. Create an account: <https://bricsguid.nia.nih.gov/portal/jsp/login.jsp>
2. Once you have an account, go to the GUID Tool – Create GUID
3. To open the 'Launch GUID Tool' you will need to have Java installed on your device
4. In order to generate a GUID, the following PHI is required ([Appendix D](#)):
 - Complete legal given (first) name of subject at birth
 - If the subject has a middle name
 - Complete legal family (last) name of subject at birth
 - Day of birth
 - Month of birth
 - Year of birth
 - Name of city/municipality in which subject was born
 - Country of birth

5.0 NCRAD Laboratory Information

5.1 Site Required Equipment

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

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

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

- Centrifuge capable of $\geq 2000 \times g$ with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:

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

5.2 Biospecimens Sent to NCRAD

Biospecimens collected include whole blood. Please refer to the below schedule for the biospecimen collection schedule:

	Week 24	Week 48
Serum	X	X
Plasma	X	X
Buffy Coat	X	X

Whole blood is collected in two different collection tubes: lavender-top EDTA tubes and plain red-top serum tubes. At Week 24 and Week 48 visits, the lavender-top EDTA tube is processed locally into plasma and buffy coat fractions, aliquoted, frozen at the study site, and then shipped to NCRAD. At Week 24 and Week 48, the plain red-top serum tube is processed locally into serum fractions, aliquoted, frozen at the study site, and then shipped to NCRAD.

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

Frozen samples are to be submitted according to the shipping methods outlined in [Section 10.2](#). Guidelines for the processing, storage location, and timing of sample collection are listed in the tables below.

5.3 Biospecimen Collection Charts

5.3.1 Biospecimen Collection for Week 24 Visit

Sample Type	Tube Type	Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
Whole blood for serum banking	Serum (Red-Top) Tube (6 mL)	1	N/A	N/A	N/A
	Serum: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	2	SERUM: 1.5 ml serum aliquots per 2.0 ml cryovial	2	Frozen
Whole blood for isolation of plasma and buffy coat	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	1	N/A	N/A	N/A
	Plasma: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	4	PLASMA: 1.5 ml plasma aliquots per 2.0 ml cryovial	Up to 4	Frozen
	Buffy Coat: 2.0 ml cryovial	1	BUFFY COAT: 0.75 ml buffy coat aliquot	1	Frozen

5.3.2 Biospecimen Collection for Week 48 Visit

Sample Type	Tube Type	Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
Whole blood for serum banking	Serum (Red-Top) Tube (6 mL)	1	N/A	N/A	N/A
	Serum: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	2	SERUM: 1.5 ml serum aliquots per 2.0 ml cryovial	2	Frozen

Whole blood for isolation of plasma and buffy coat	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	1	N/A	N/A	N/A
	Plasma: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)	4	PLASMA: 1.5 ml plasma aliquots per 2.0 ml cryovial	Up to 4	Frozen
	Buffy Coat: 2.0 ml cryovial	1	BUFFY COAT: 0.75 ml buffy coat aliquot	1	Frozen

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.

6.0 Specimen Collection Kits, Shipping Kits, and Supplies

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD, the Blood Supplemental Supply Kit, and the Frozen Shipment Kit; 2) clinical lab supplies (with the exception of dry ice and equipment supplies listed in [Section 5.1](#)). These materials include blood tubes, pipettes, boxes for serum/plasma/buffy coat aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, Site and ADCS ID Labels, Collection and Aliquot Tube Labels will all be provided by NCRAD. Details regarding the blood are found in this Manual of Procedures. Collection and Aliquot Tube Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to [Section 7.1](#).

6.1 Specimen Collection Kit Contents

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

T2 PROTECT AD Blood Collection Kit-Weeks 24 and 48

Quantity	Blood Collection Kit Components
1	Serum Red Top Blood Collection Tube (6 mL)

1	EDTA Lavender Top Blood Collection Tube (10 mL)
3	Cryovial tube (2.0 mL) with lavender cap
1	Cryovial tube (2.0 mL) with clear cap
2	Cryovial tube (2.0 mL) with red cap
2	Cryovial tube (2.0 mL) with blue cap
3	Disposable graduated transfer pipette
10	Pre-printed Collection and Aliquot Tube Label
2	Pre-printed Kit Number Label
3	Labels for Handwritten Site and ADCS ID
1	Microcentrifuge box (25-slot)

Blood Supplemental Supply Kit

Quantity	Blood-Based Supplemental Supply Kit Components
5	Serum (Red-Top) Blood Collection Tube (6 ml)
5	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
10	Cryovial tube (2.0 ml) with lavender cap
10	Cryovial tube (2.0 ml) with red cap
10	Cryovial tube (2.0 ml) with blue cap
5	Cryovial tube (2.0 ml) with clear cap
20	Disposable graduated transfer pipette
10	Labels for handwritten Site and ADCS ID
5	Microcentrifuge box (25-slot)

T2 PROTECT AD Frozen Shipping Kit

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

6.2 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 kits.iu.edu/t2 to request additional kits and follow the prompts to request the desired supplies. Options include ordering a specific number of kits; we are also including the option of simply ordering the desired amount of extra supplies.

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

7.0 Blood Collection and Processing Procedures

7.1 Labeling Samples

Important Note

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

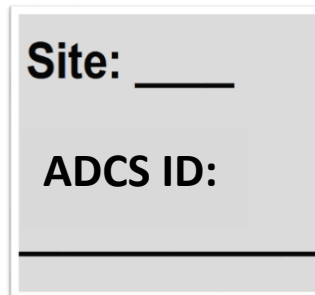
1. Plain Red Top Serum Blood Collection Tube (6 ml)
2. EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Buffy Coat and Plasma

Label Type Summary

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



The **Kit Number Labels** do not indicate a specimen type, but are affixed on the Biological Sample and Shipment Notification Forms and on specific packing materials.



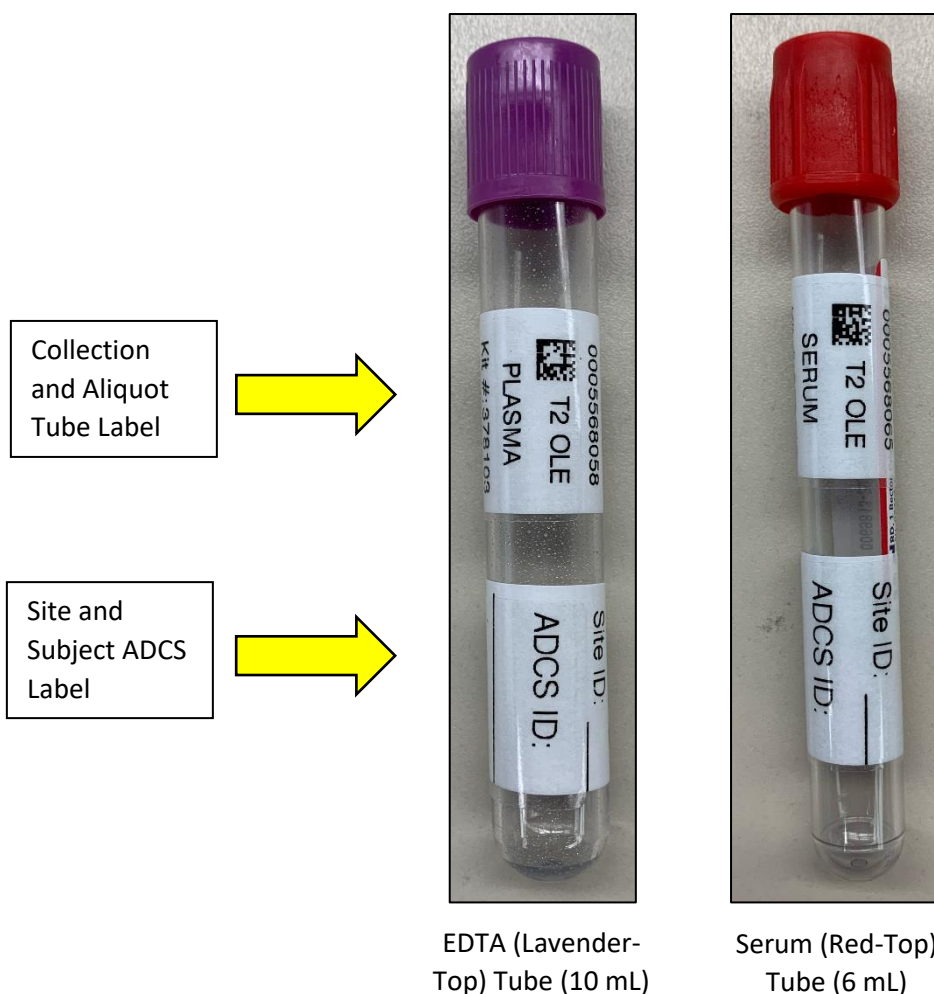
The **Site and ADCS Labels** are placed on all collection tubes.



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

****Important Note****

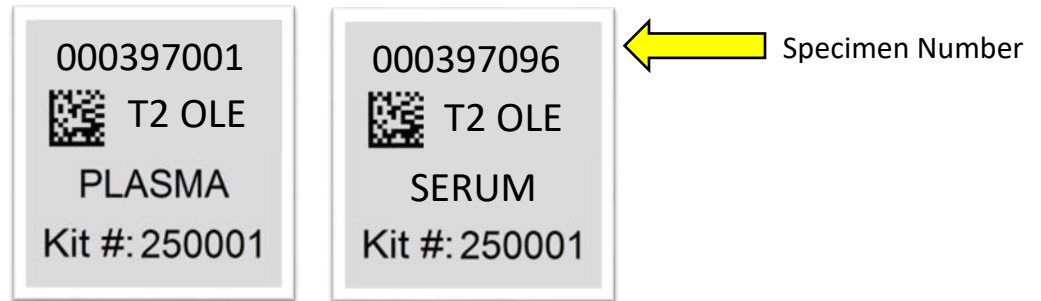
Each collection tube will contain two labels: the Collection and Aliquot Tube Label and the Site and ADCS ID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten Site and ADCS label.



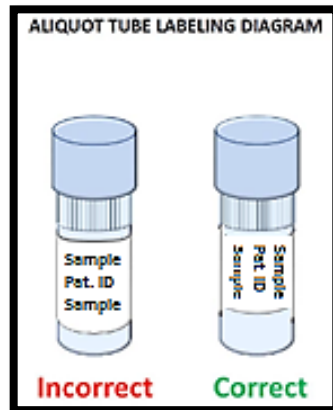
In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

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

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



- Using a fine point marker, fill-in and place the Site and ADCS Labels on the collection tubes only (Serum, EDTA) **BEFORE** sample collection, processing, or freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place Site and ADCS ID labels on any cryovials.
- 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.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see labeling diagram below).
- Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label.



- If there are any unused cryovials, please do not send the empty cryovials to NCRAD. These unused cryovials (ensure labels are removed) can be saved as part of a supplemental supply at your site or the cryovials can be disposed of per your site's requirements.

7.2 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/resources_T2_PROTECT_AD.html

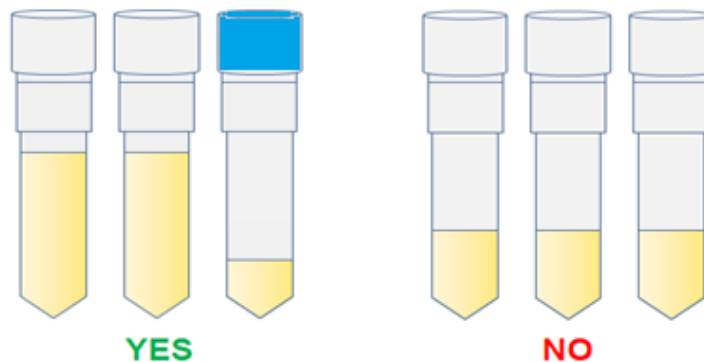
- Plasma and Buffy Coat Processing and Aliquoting
- Serum Processing and Aliquoting
- Frozen Shipping

7.3 Filling Aliquot Tubes (Plasma and Serum)

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

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

Aliquot the remaining biologic material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 3.6 ml of sample is obtained, you should fill 2 cryovial tubes each with 1.5 ml, and one additional cryovial tube with the remaining 0.6 ml for plasma and serum.

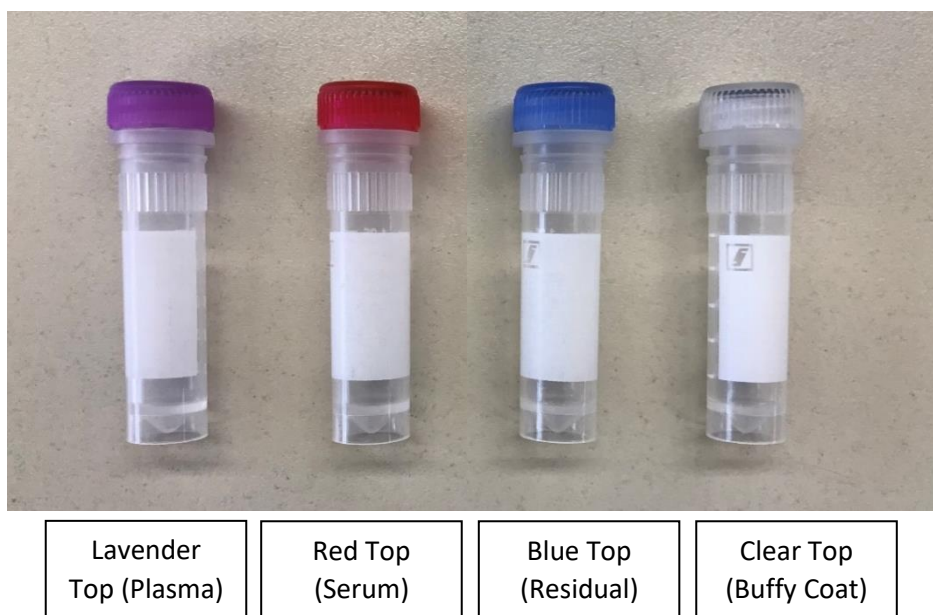


Please note: It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume (anything under 1.5 ml for plasma or

serum). Please record the specimen number and volume of the residual aliquot on the Biological Sample and Notification Form.

To assist in the preparation and aliquoting of samples, colored caps and cap stickers are used for the cryovial tubes. The chart below summarizes the association between cap color and type of cryovial.

Cap Color	Sample Type
Lavender Cap	Plasma/Plasma-PK
Red Cap	Serum
Blue Cap	Residual
Clear Cap	Buffy Coat



7.4 Serum (Red-top) Tube (6 mL) for Serum

Whole Blood Collection for Isolation of Serum: Serum (Red-Top) Tube (6 ml) (for processing of serum aliquots). One Red-Top tube is collected at Weeks 24 and 48.

1. Set centrifuge 4°C to pre-chill before use.
2. Place completed Site and ADCS ID Label and Collection and Aliquot **"SERUM"** Tube Labels on the Plain Red-Top Serum Blood Collection Tube. Place pre-printed Collection and Aliquot **"SERUM"** Tube Labels on the (2) 2.0 ml cryovial

tubes with red caps and (1) 2.0 ml cryovial with blue cap (if necessary, for residual).

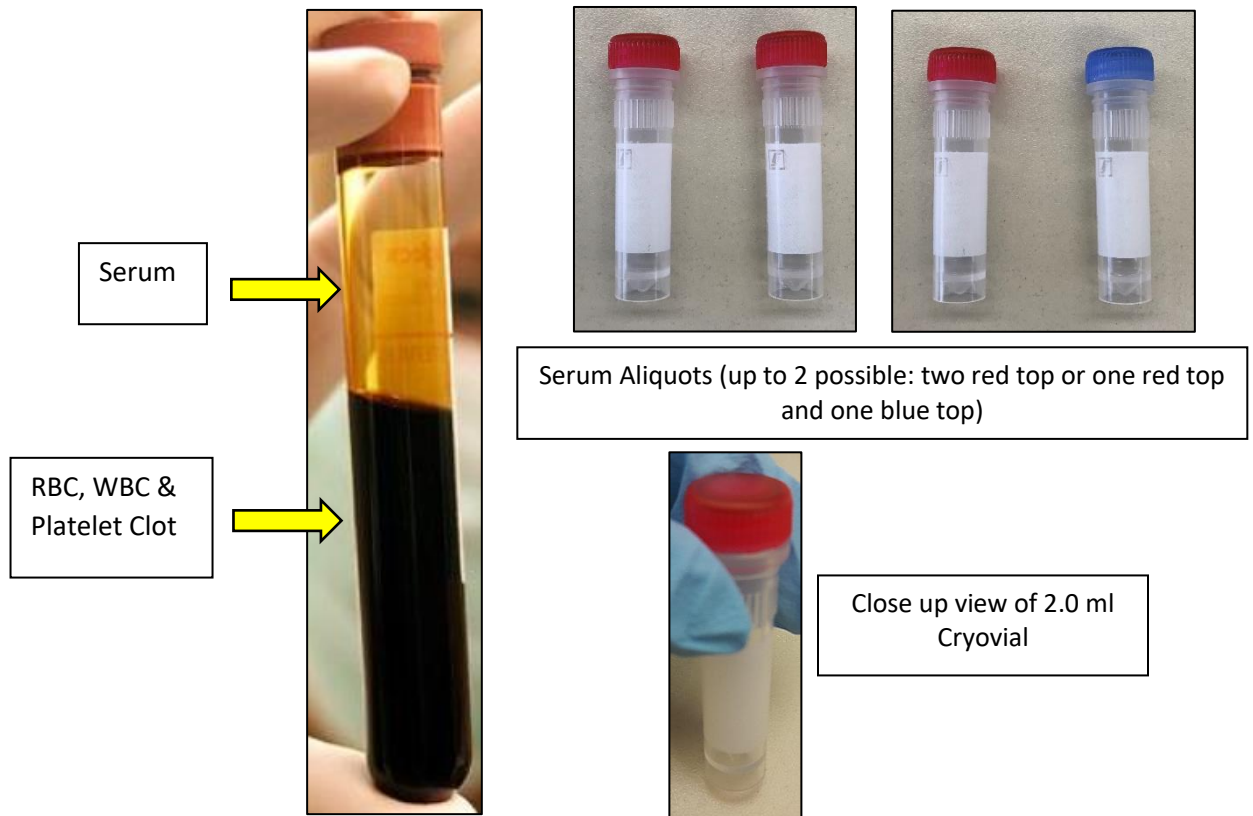
3. Using a blood collection set and a holder, collect blood into **Plain Red-Top Serum Blood Collection Tubes (6 ml)** using your institution's recommended procedure for standard venipuncture technique

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 tube.
 - d. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into each tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 5 ml of blood into the tube.
 - a. If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional Serum tube at this time. Process blood obtained in existing Serum tube.
 5. **CRITICAL STEP:** Immediately after blood collection, **gently** invert/mix (180 degree turns) each tube 5 times.
 6. **CRITICAL STEP:** Allow blood to clot at room temperature by placing it upright in a vertical position in a tube rack for 30 minutes. If sample is not clotted allow it to set up to 60 minutes to clot. Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
 7. After 30 minutes of clotting, centrifuge the collection tube for 10 minutes at 2000 x g at 4°C. **It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum separation (see worksheet in [Appendix A](#) to calculate RPM)**
 - a. Equivalent rpm for spin at 2000 x g
 - b. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form [Appendix B](#).
 - c. Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.

d. Record time aliquoted on the Biological Sample Shipment and Notification Form

8. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the wall. Using a disposable pipette, transfer serum into the pre-labeled cryovials with the red caps. Aliquot 1.5 ml per cryovial (total vials= up to two with 1.5 ml each or one with 1.5 mL and one residual with <1.5 ml). Be sure to only place **serum** in cryovials labeled with the "SERUM" label and red caps. If there is extra serum left, use 1 extra blue-cap cryovial provided for another <1.5 ml aliquot of serum and label as appropriate. **If a residual aliquot (<1.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**



9. Place the labeled cryovials in a 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 and Shipment Notification Form.

Serum Preparation (6ml Red Top Tube)

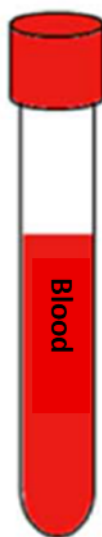


Step One



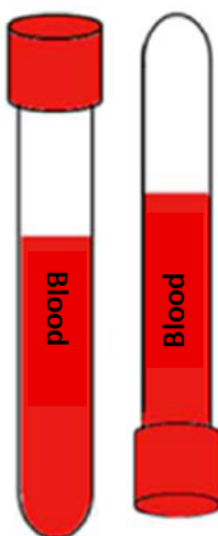
- Store tubes at room temperature.
- Label tubes with pre-printed subject labels prior to blood draw.

Step Two



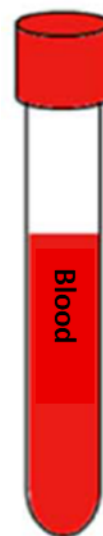
- Collect blood in Serum Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



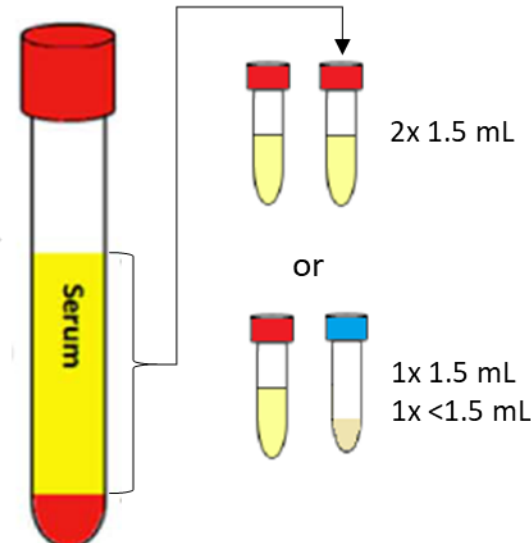
- Immediately after blood draw, invert tubes 5 times to mix samples.

Step Four



- Allow blood to clot for 30 minutes.
- Within 60 minutes of blood draw, centrifuge samples at 2000 x g for 10 minutes at 4°C

Step Five



- Label cryovial tubes with preprinted labels.
- Aliquot 1.5 ml into each red-cap cryovial tube.
- If any residual remains, aliquot into a blue-cap cryovial and note the residual on the Biological Sample and Shipment Notification Form.
- Samples need to be spun, aliquoted, and frozen within 2 hours from time of collection.
- Store plasma aliquots upright at -80°C until shipment.

7.5 EDTA (Lavender-Top) Blood Collection Tube (10 mL) for Plasma and Buffy Coat

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

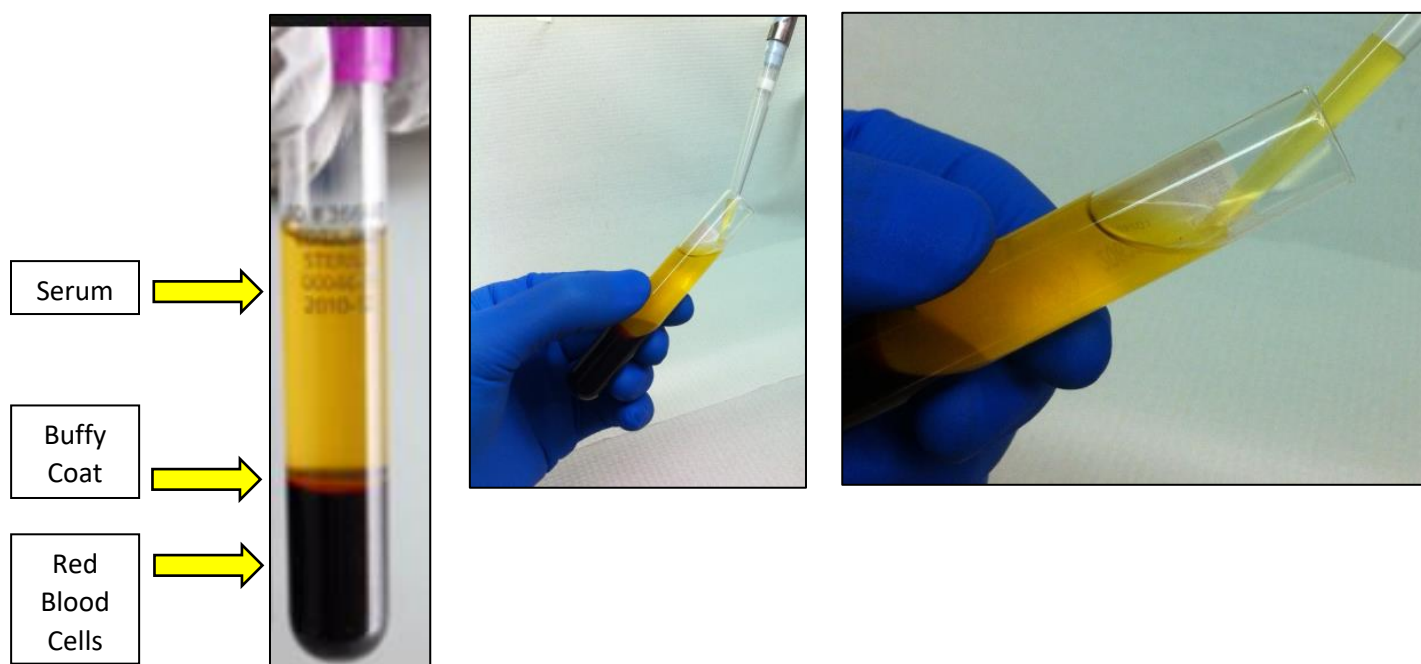
1. Set centrifuge to 4°C to pre-chill before use.
2. Place completed Site and ADCS ID Label and pre-printed “**PLASMA**” Collection and Aliquot Tube Label on the lavender-top EDTA tube. Place pre-printed “**PLASMA**” Collection and Aliquot Tube Labels on the three 2.0 ml cryovial tubes with lavender caps. Place pre-printed “**BUFFY COAT**” Collection and Aliquot Tube Label on the (1) 2 ml cryovial with a clear cap.
3. Please ensure that aliquots are kept in numerical order (by specimen number) throughout the aliquoting and shipping process.
4. Using a blood collection set and a holder, collect blood into the **EDTA (Lavender-Top) Blood Collection Tube (10 ml)** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

- a. Place 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 tube.
 - d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
 - a. If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.
 6. **CRITICAL STEP:** Immediately after blood collection, **gently** invert/mix (180 degree turns) the EDTA tube 8-10 times.

7. **CRITICAL STEP: Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.**
 - a. Preferably within 30 minutes of blood collection, centrifuge balanced tubes for 10 minutes at 2000 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.**
 - b. Equivalent rpm for spin at 2000 x g
 - c. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
 - d. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
 - e. Record time aliquoted on the Biological Sample and Shipment Notification Form.

8. Remove the plasma, being careful not to agitate the packed red blood cells at the bottom of the collection tube. Tilt the tube and placing the disposable pipette tip along the lower side of the wall without touching the pellet (buffy coat) so that plasma is not contaminated (see below). Transfer plasma into the pre-labeled cryovials. Aliquot 1.5 ml per cryovial (3 vials with 1.5 ml each). Be sure to only place **plasma** in cryovials labeled with “PLASMA” labels. Take caution not to disturb the red blood cells at the bottom of the tube. If there is extra plasma left, use 1 extra cryovial with blue cap provided for another <1.5 ml aliquot of plasma. **If a residual aliquot (<1.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**



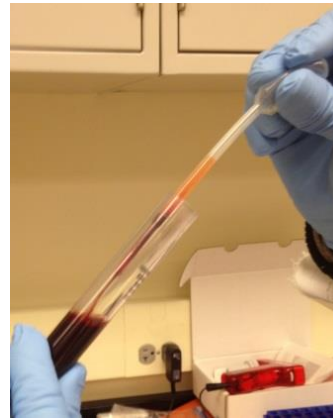
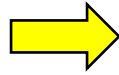
NOTE: When pipetting plasma from the plasma tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.



Up to 4 cryovials possible: three lavender top and one blue top. Lavender top cryovials have 1.5ml plasma and blue top cryovial used if <1.5ml remains.

9. Place the labeled cryovials in the cryobox and place upright on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.
10. After plasma has been removed from the 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 labeled cryovial with clear cap using a disposable graduated micropipette. All of the buffy coat will be placed into one cryovial. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovial with the clear cap and "BUFFY COAT" lab

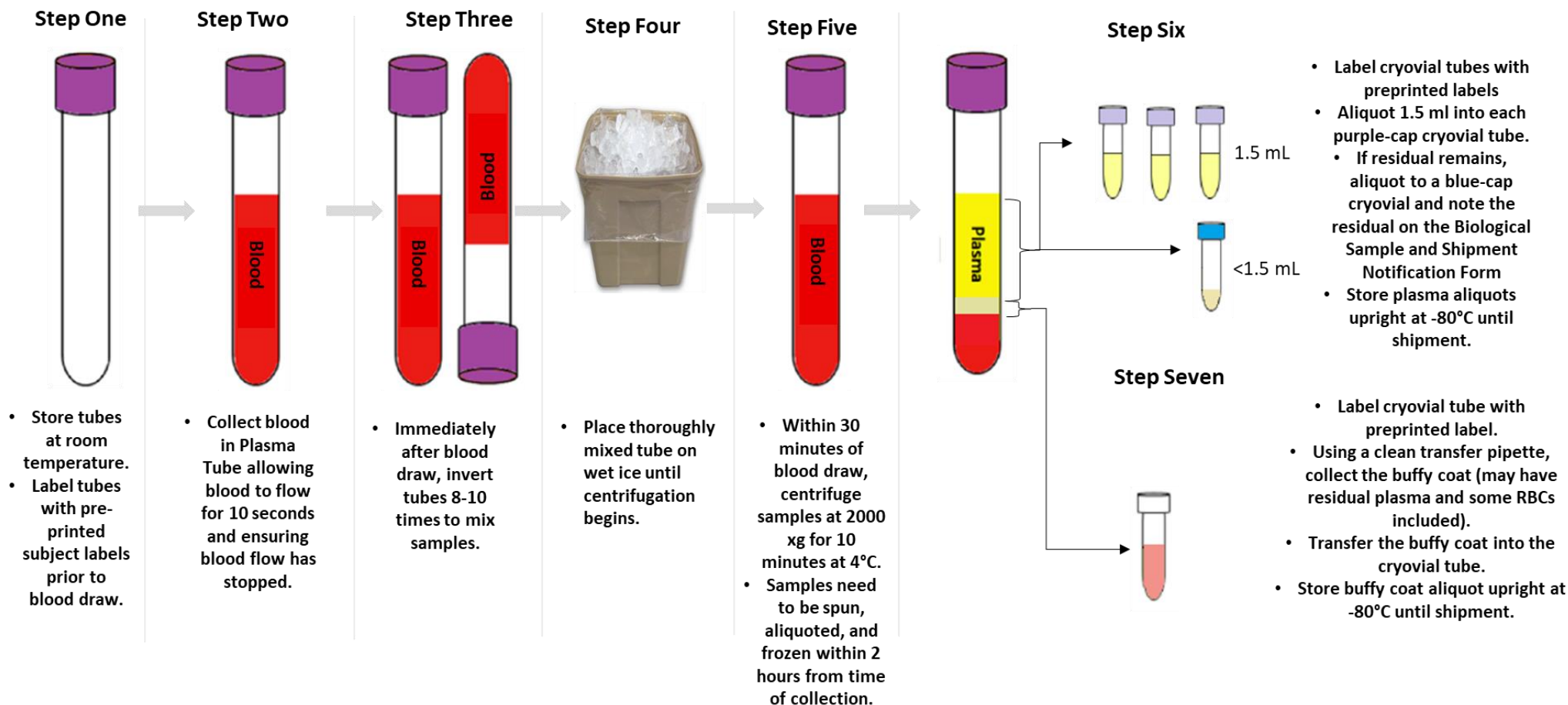
Buffy Coat
layer (mixed
with RBCs)



Buffy Coat Aliquot
(Please use CLEAR
CAP cryovial)

11. Dispose of collection tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.
12. Place the labeled cryovial in a cryobox and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples upright at **-80°C until shipped** to NCRAD on dry ice.

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



8.0 Sample Redraws

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

If challenges arise during the blood draw process, it is advised that the phlebotomist discontinue the draw. Attempt to process and submit any blood-based specimens that have already been collected to NCRAD.

Redraws will be scheduled for samples submitted to NCRAD.

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.

1. If the biospecimens at a scheduled visit **are partially** collected:
 - a. Attempt to process and submit any samples that were able to be collected during the visit.
 - b. Document difficulties on the 'Biological Sample and Shipment Notification Form' prior to submission to NCRAD.
 - i. Indicate blood draw difficulties at the bottom of the 'Biological Sample and Shipment Notification Form' within the "Notes" section.
 - ii. Complete the 'Biological Sample and Shipment Notification Form' with tube volume approximations and number of aliquots created.
 - c. Contact a NCRAD coordinator and alert them of the challenging blood draw.
2. If the biospecimens at a scheduled visit **are not** collected:
 - a. Contact the T2 PROTECT AD Project Manager and a NCRAD coordinator to alert them of the challenging blood draw or circumstances as to why biospecimens were not collected.

Schedule participant for a re-draw visit as quickly as possible.

9.0 Packaging and Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your University, please contact NCRAD with questions and information regarding resources.

Sample Type	Processing/Aliquoting	Tubes to NCRAD	Ship
Whole blood (Lavender-Top EDTA tube) for isolation of plasma and buffy coat	1.5 ml plasma aliquots per 2.0 ml cryovials	Up to 4	Frozen
	0.75 ml buffy coat aliquot per 2.0 ml cryovial	1	Frozen
Whole blood (Red-Top tube) for isolation of serum	1.5 ml serum aliquots per 2.0 ml cryovials	Up to 2	Frozen

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.

10.1 Frozen Batch Shipping Instructions

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

*** Packing and Labeling Guidelines ***

- The primary receptacle (frozen cryovials) must be leak proof and must not contain more than 1L total.
- The secondary packaging (biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (within the cryovial box containing the frozen cryovials) and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
 - ✓ Sender's name and address
 - ✓ Recipient's name and address
 - ✓ Responsible Person
 - ✓ The words "Biological Substance, Category B"
 - ✓ UN3373
 - ✓ Class 9 label including UN 1845, and net weight of dry ice contained



1. A sample shipment to NCRAD should be initiated when a study site has five (5) cryoboxes of samples or every three (3) months, whichever is sooner.
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 Completed Biological Sample and Shipment Form to the email ([Appendix B](#)). If email is unavailable, please call NCRAD and do not ship until you have notified NCRAD coordinators of the shipment in advance.

4. Place all labeled and frozen plasma, serum, and buffy coat aliquots in a cryobox.
5. Label the outside of the cryobox with the kit number label and place in a clear biohazard bag. Do not remove absorbent material found in the bag and seal according to the instructions on the bag.



6. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam shipping container.
7. Place the biohazard bag into the provided Styrofoam-lined shipping container on top of the dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container. Layer dry ice and cryoboxes as necessary.
8. The inner Styrofoam shipping container must contain approximately 30-45 lbs (or ~21kg) of dry ice. The dry ice should entirely fill the inner box to ensure the frozen state of the specimens.

Full Shipping Container with
Batched Samples and Dry Ice



9. Replace the lid of the Styrofoam container. 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. Be sure to NOT completely seal the outer cardboard box with tape, as the dry ice needs to vent.

10. Complete the FedEx return airbill with the following information:
 - Section 1, “From”: fill in your name, address, phone number, and Site FedEx Account Number.
 - Section 2, “Your Internal Billing Reference”: add any additional information required by your site.
 - 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.
 - Section 7, “Payment”, check third party and bill transportation costs to the T2 PROTECT AD study FedEx account number.
11. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
 - Your name and return address
 - Net weight of dry ice in kg (must match amount on the airbill)
 - Consignee name and address:

NCRAD
IU School of Medicine
351 W. 10th St TK-217
Indianapolis, IN 46202
 - Do not cover any part of this label with other stickers, including pre-printed address labels.
12. Apply all provided warning labels and the completed FedEx return airbill to the outside of the package, taking care not to overlap labels.
13. Hold packaged samples in -80C freezer until time of FedEx pick-up/drop-off.
14. Specimens should be sent to the address below via FedEx Priority Overnight. Frozen specimens should be sent **Monday through Wednesday** to avoid any potential shipping delays. FedEx does not replenish dry ice if shipments are delayed or held over the weekend.

NCRAD
IU School of Medicine
351 W. 10th St. TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839
15. 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.

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.

In addition to tracking and reconciliation of samples, the condition and number 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.

10.0 Data Queries and Sample Reconciliation

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

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

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

11.0 Appendices List

Appendix A: Rate of Centrifuge Worksheet

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

Submitter Information

Name:

Site:

Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor: ☐

Swing Bucket Rotor: ☐

Radius of Rotation (mm):

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

Calculating RPM from G-Force:

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

RCF = Relative Centrifugal Force (G-Force)

RPM = Rotational Speed (revolutions per minute)

R= Centrifugal radius in mm = distance from the center of the turning axis to the bottom of centrifuge

Comments:

Please send this form to NCRAD Study Coordinator

317-321-2003 (Fax)

alzstudy@iu.edu

Appendix B: Biological Sample and Shipment Notification Form

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

To: Kelley Faber Email: alzstudy@iu.edu Phone: 1-800-526-2839			
General Information:		FedEx tracking #: _____	
From: _____	Date: _____		
Phone: _____	Email: _____		
Study: T2 PROTECT AD OLE GUID: _____ Kit #: _____ Visit: Week 24 Week 48 Early Termination Site ID: _____ ADCS PTID #: _____ Sex: M F Year of Birth: _____		KIT BARCODE	
Blood Collection:			
1. Date Drawn: _____ [MM/DD/YY]		2. Time of Draw: _____ [HHMM]	
3. Date subject last ate: _____ [MM/DD/YY]		4. Time subject last ate: _____ [HHMM]	
Blood Processing:			
Serum (Red-top) Tube (6 mL)		Plasma & Buffy Coat (Lavender-top) Tube (10 mL)	
Time spin started: _____ [HHMM]		Time spin started: _____ [HHMM]	
Duration of centrifuge: _____ Minutes		Duration of centrifuge: _____ Minutes	
Temp of Centrifuge: _____ °C Rate of centrifuge: _____ x g		Temp of Centrifuge: _____ °C Rate of centrifuge: _____ x g	
Original volume drawn (1 x 6 mL tube): _____ mL		Original volume drawn (1 x 10 mL tube): _____ mL	
Time aliquoted: _____ [HHMM]		Time aliquoted: _____ [HHMM]	
Number of 1.5 mL serum aliquots created (red cap): _____		Number of 1.5 mL plasma aliquots created (lavender cap): _____	
If applicable, volume of residual serum aliquot (less than 1.5 mL in blue cap): _____ mL		If applicable, volume of residual plasma aliquot (less than 1.5 mL in blue cap): _____ mL	
If applicable, specimen number of residual serum aliquot (last four digits): _____		If applicable, specimen number of residual plasma aliquot (last four digits): _____	
Time aliquots placed in freezer: _____ [HHMM]		Time aliquots placed in freezer: _____ [HHMM]	
Storage temperature in freezer: _____ °C		Storage temperature in freezer: _____ °C	
		Buffy coat aliquot created (clear cap, one per 10 mL EDTA tube)	_____ mL
Notes: _____			

Appendix C. GUID Demographics Form

Please be certain to collect the following demographic information to generate a Global Unique Identifier:

1. Complete legal given (first) name of subject at birth: _____
2. Complete additional (middle) name or names at birth: _____
3. Complete legal family (last) name of subject at birth: _____
4. Suffix: _____
5. Date of Birth: _____
6. Name of city/municipality in which subject was born: _____
7. Country of birth: _____