

Head-to-head Evaluation of tau tracers in Alzheimer's Disease

in collaboration with

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

Biofluid Collection, Processing and Shipment

Manual of Procedures

Version 1.0

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1.0 ABBREVIATIONS

AD	Alzheimer's Disease
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
HEAD	Head-to-head Evaluation of tau tracers in Alzheimer's Disease
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
SST	Serum Separator Tube
UPS	United Parcel Service

2.0 PURPOSE

The collection of biofluids is an important part of the Head-to-head Evaluation of tau tracers in Alzheimer's Disease (HEAD) Study. The purpose of this manual is to provide study staff (PIs, study coordinators, phlebotomists) at the various study sites with instructions for collection and submission of biological samples for HEAD study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

The following samples will be sent to NCRAD:

- Serum
- Plasma
- Buffy Coat (DNA Extraction)
- Whole Blood for banking
- Whole Blood for RNA

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

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



3.0 NCRAD INFORMATION

3.1 NCRAD Contacts

Tatiana Foroud, PhD, NCRAD Leader

Phone: 317-274-2218

Kelley Faber, MS, CCRC, Project Manager

Phone: 317-274-7360

Email: kelfaber@iu.edu

Colleen Mitchell, Laboratory Manager

Phone: 317-278-9016

Email: mitchebcm@iu.edu

Diont'e Keys, BS, CCRP Study Coordinator

Phone: 317-274-7546

Email: dlkeys@iupui.edu

General NCRAD Contact Information

Phone: 1-800-526-2839

Email: alzstudy@iu.edu

Website: www.ncrad.org

HEAD Study Specific Webpage: https://ncrad.org/resource_HEAD.html

Sample Shipment Mailing Address

HEAD at NCRAD

Indiana University School of Medicine

351 West 10th Street

TK-217

Indianapolis, IN 46202

3.2 Hours of Operation

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

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

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

3.3 Holiday Schedules

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

3.4 Holiday Observations

Date	Holiday
January 1	New Year's Day
3 rd Monday in January	Martin Luther King, Jr Day
4 th Monday in May	Memorial Day
June 19	Juneteenth (observed)
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.**

- Please note that courier services may observe a different set of holidays.
- Please be sure to verify shipping dates with your courier prior to any holiday.
- **Although rarely occurs, weekend/holiday delivery must be arranged in advance with NCRAD staff.**

4.0 NCRAD LABORATORY COLLECTION

4.1 Site Required Equipment

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

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquet

- Alcohol Prep Pad
- Gauze Pad
- Bandage
- Butterfly needles and hub
- Microcentrifuge tube rack
- Sharps bin and lid
- Wet Ice Bucket
- Wet ice
- Dry ice

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

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

In order to ship specimens, you must provide:

- Dry ice (approximately 45 lbs per shipment)

4.2 Biofluid Collection Schedules

HEAD Biospecimen Collection Schedule:

Specimen Type	Baseline Visit	18 Month Visit
Whole Blood for RNA	X	X
Serum	X	X
Plasma	X	X
DNA	X	X
Whole Blood for banking	X	X

Whole blood is collected in four types of tubes (10ml Serum Tube, 10ml lavender-top EDTA tube, 6ml EDTA tube, and 2.5ml PAXgene™ Blood RNA tube). The 10ml red-top serum tube is processed locally into serum and then aliquoted, frozen at the study site and shipped to NCRAD. The 10ml EDTA tubes are processed locally into plasma and buffy coat fractions. They are then aliquoted, frozen at the study site, and shipped to NCRAD.

The 6ml EDTA tube and PAXgene™ Blood RNA tube are frozen locally without further processing and shipped to NCRAD.

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

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

4.3 Biofluid Collection Charts

4.3.1 Biofluid Collection for Baseline and 18 M Visits

Draw Tube Order	Sample Type	Tube Type	Number of Tubes Supplied in Kit	Aliquot Volume	Tubes to NCRAD	Ship
1	Whole Blood for Transcriptome Analysis	PAXgene™ Blood RNA Collection Tube (2.5 ml)	1	N/A	1	Frozen
2	Whole blood for isolation for serum	Serum Separator (Red-Top) Blood Collection Tube (10ml)	1	N/A	N/A	N/A
		Serum: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	4	1.5 ml serum aliquot per 2.0 ml cryovial (red cap)	Up to 4	Frozen
3	Whole blood for isolation of plasma & buffy coat (for DNA extraction)	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	5	N/A	N/A	N/A
		PLASMA: 2.0 ml cryovials with purple cap (residual volume placed in 2.0 ml cryovial with blue cap)	17	1.5 ml plasma aliquot per 2.0 ml cryovial (purple cap)	Up to 17	Frozen
		BUFFY COAT: 2.0 ml cryovial	5	1 ml buffy coat aliquot per 2.0 ml cryovial (gray cap)	5	Frozen
4	Whole blood for future analysis	EDTA (Lavender-Top) Blood Collection Tube (6 ml)	1	N/A	1	Frozen

If a sample is not obtained at a particular visit, it should be recorded in the notes section of the **Biological Sample and Shipment Notification Form** (see [Appendix B](#)). Submit a copy to NCRAD with a reason provided for the omission and track it as a protocol deviation.

5.0 SPECIMEN COLLECTION KITS, SHIPPING KITS, AND SUPPLIES

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD, the Blood Supplemental Supply Kit, the Frozen Shipment Supply Kit 2) clinical

lab supplies (with the exception of dry ice and equipment supplies listed in [Section 4.1](#)). The provided materials include blood tubes, pipettes, boxes for serum, plasma/buffy coat aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, HEAD ID Labels, and Collection Tube Labels will all be provided by NCRAD. Details regarding the blood kits are found in this Manual of Procedures. Collection Tube and Cryovial 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.

HEAD Blood-Based Kits

Quantity	HEAD Blood-Based Kit Components
1	Serum red top tube (10 ml)
5	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
1	EDTA (Lavender-Top) Blood Collection Tube (6 ml)
1	PAXgene Blood RNA Tube (2.5ml)
3	2ml Robotic Freezer Cryovials– RED
16	2ml Robotic Freezer Cryovials - PURPLE
2	2ml Robotic Freezer Cryovials - BLUE
5	2ml Robotic Freezer Cryovials - GRAY
5	Disposable graduated transfer pipette (3ml)
1	50ml conical
8	Pre-printed Collection Tube Label
26	Cryovial Label
3	Pre-printed Kit Number Label
8	Labels for handwritten PT ID
2	Resealable bubble wrap tube pouches
1	Resealable bag
1	Cryovial box (holds up to 48 cryovials)
1	Plastic Biohazard bag with absorbent sheet (small)

Blood-Based Supplemental Supply Kit

Quantity	Blood-Based Supplemental Supply Kit Components
2	Serum red top tube (10 ml)
10	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
2	PAXgene Blood RNA Tube (2.5 ml)
2	EDTA (Lavender-Top) Blood Collection Tube (6 ml)
10	Disposable graduated transfer pipette (3ml)
2	50ml Conical Tube
16	Labels for handwritten HEAD ID
4	Bubble wrap tube sleeve for frozen blood tubes
2	Resealable bag
2	Cryovial box (holds up to 48 cryovials)
2	Plastic Biohazard bag with absorbent sheet (large)
32	2ml Robotic Freezer Cryovials - PURPLE
10	2ml Robotic Freezer Cryovials - GRAY
6	2ml Robotic Freezer Cryovials- RED
4	2ml Robotic Freezer Cryovials - BLUE
2	UPS Blue Dry Ice Sticker
2	UN3373 Sticker
2	Fragile Label
2	Biohazard Label

HEAD Frozen Blood Shipping Supply Kit

Quantity	Frozen Shipping Kit Components
5	Plastic Biohazard bag with absorbent sheet (large)
1	Resealable bag
1	Shipping box/Styrofoam container
1	UPS Blue Dry Ice Sticker
1	UN3373 Sticker
1	Fragile Label
1	Biohazard Label

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module
By Request	Serum red top tube (10 ml)
By Request	PAXgene Blood RNA Tube (2.5 ml)
By Request	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
By Request	EDTA (Lavender-Top) Blood Collection Tube (6 ml)
By Request	Disposable graduated transfer pipette (3ml)
By Request	50ml conical

By Request	Labels for handwritten HEAD ID
By Request	Bubble wrap tube sleeve for frozen blood tubes
By Request	Resealable bag
By Request	Cryovial box (holds up to 48 cryovials)
By Request	Plastic Biohazard bag with absorbent sheet (large)
By Request	UN3373 sticker
By Request	Dry Ice label
By Request	Fragile labels
By Request	Shipping box
By Request	2ml Robotic Freezer Cryovials - PURPLE
By Request	2ml Robotic Freezer Cryovials - GRAY
By Request	2ml Robotic Freezer Cryovials– RED
By Request	2ml Robotic Freezer Cryovials - BLUE

5.2 Kit Supply to Study Sites

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

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

6.0 BLOOD COLLECTION AND PROCESSING PROCEDURES

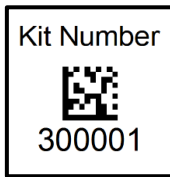
Important Note

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

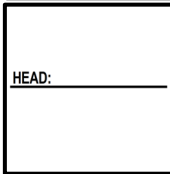
6.1 Labeling Samples

Label Type Summary

1. Kit Number Label
2. HEAD ID Label
3. Collection Tube Label
4. Cryovial Label



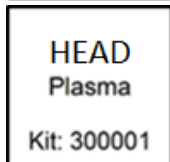
The **Kit Number Labels** do not indicate a specimen type, but are affixed on the Biological Sample and Shipment Notification Form and on specific packing materials. This label ties together all specimens collected from one subject at one visit.



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



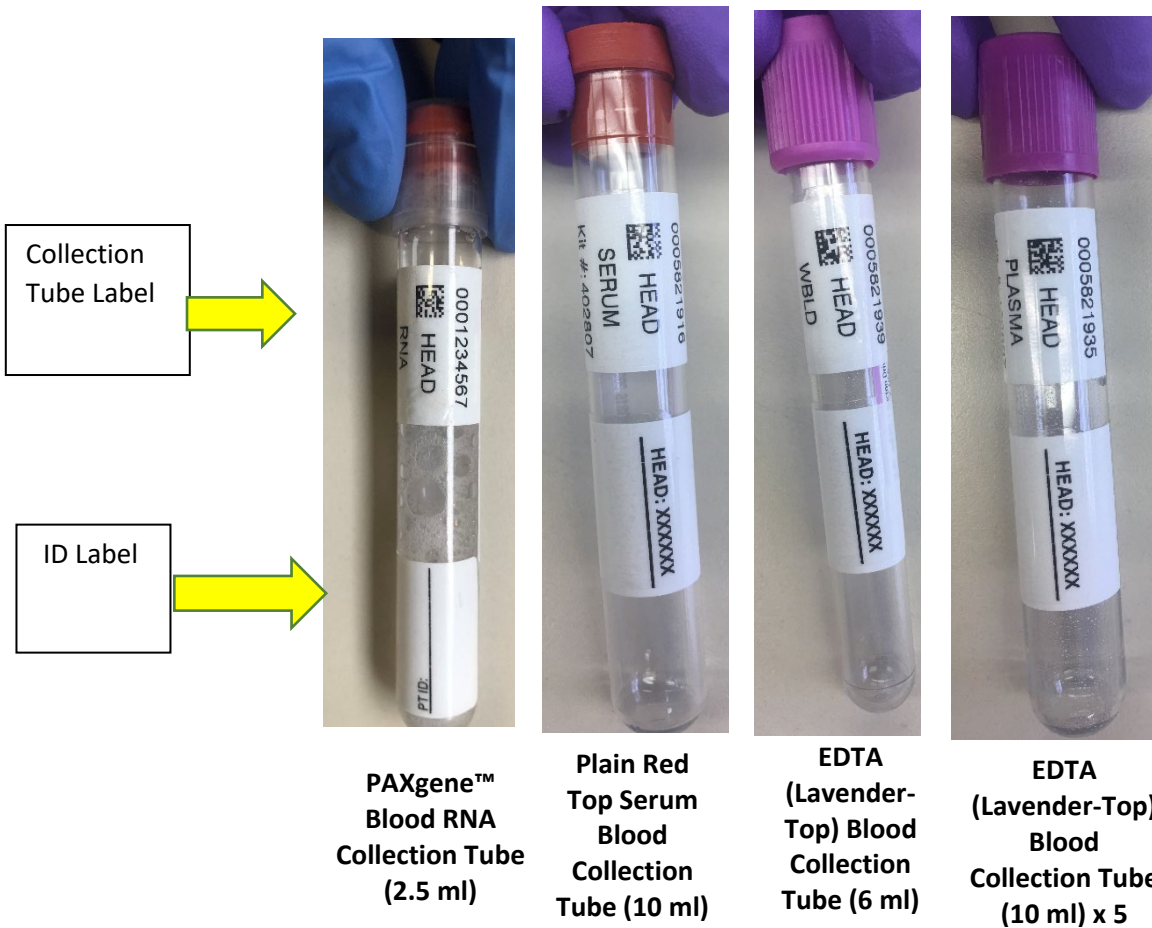
The **Collection Tube Labels** for blood derivatives are placed on all collection tubes.



Place one **Cryovial Label** on each cryovial.

****Important Note****

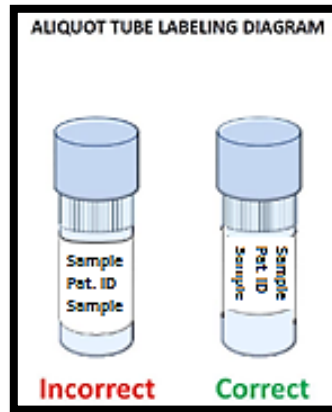
Each collection tube will contain two labels: the Collection Tube Label and the HEAD ID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten HEAD ID Label below.



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

- Place blood collection and aliquot labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing, or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Using a fine point permanent marker, fill-in the HEAD ID Labels and place on the collection tubes only **BEFORE** sample collection, processing, or freezing. These labels are in addition to the Collection Tube Labels. **DO NOT** place HEAD ID labels on any cryovials.
- The Collection 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.

6.2 Filling Aliquot Tubes (Serum and Plasma)

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

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

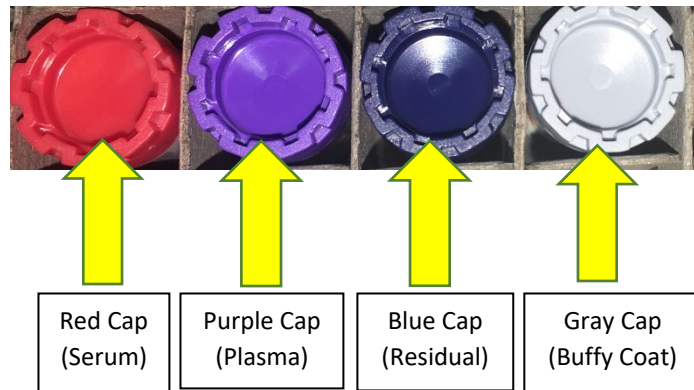
Aliquot the remaining biological material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 3.6 ml of sample is obtained, you should fill 2 cryovials each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



Note: It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume (anything under 1.5 ml). Please highlight that the aliquot contains a small volume by utilizing the blue cryovial cap provided in each kit. Please record the specimen number and volume of the residual aliquot on the Biological Sample and Notification Form.

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

Cap Color	Sample Type
Red Cap	Serum
Purple Cap	Plasma
Blue Cap	Residual (plasma)
Gray Cap	Buffy Coat



6.3 2.5 ml PAXgene™ Blood RNA Tube

See training video for blood collection:
<https://www.labtube.tv/video/MTAxMzc2>.

See training video for Freezing Blood in the PAXgene Blood RNA Tube:
<https://www.labtube.tv/video/MTAxMzc1>.

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

1. Place filled-out Site and HEAD ID Label and Collection and Aliquot “**Whole Blood**” Tube Label on the PAXgene™ tube (2.5 ml) prior to blood draw; no processing is required for this tube. **The single tube is to be shipped to NCRAD frozen, without processing at the collection site.**
2. Using a blood collection set and a holder, collect blood into the **PAXgene™ Blood RNA Tube (2.5 ml)** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

- a. Place participant's arm in a downward position.
 - b. Hold tube in a vertical position, below the participant’s arm during blood collection.
 - c. Release tourniquet as soon as blood starts to flow into tube.
 - d. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The PAXgene™ Blood RNA Tube (2.5 ml) with its vacuum is designed to draw 2.5 ml of blood into the tube.
 5. **Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene™ Blood RNA Tube (2.5 ml) 8 – 10 times.**
 6. Place the PAXgene™ Blood RNA tube (2.5 ml) upright in a **WIRE** rack and transfer the PAXgene™ Blood RNA tube (2.5 ml) to a **-80°C freezer**. Keep the **PAXgene™ Blood RNA Tube (2.5 ml) in -80°C freezer** for storage until you ship on dry ice to NCRAD. Complete remainder of the Biological Sample and Shipment Notification Form ([Appendix B](#)).

RNA Preparation (2.5ml PAXgene™ Tube)

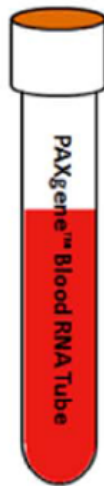


Step One



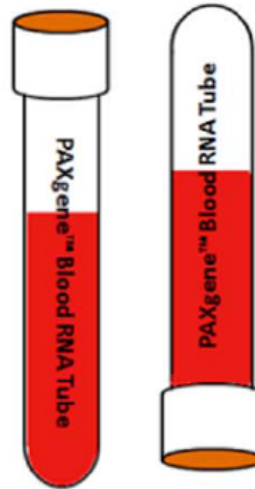
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



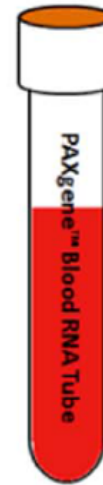
- Collect blood in PAXgene™ tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

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.



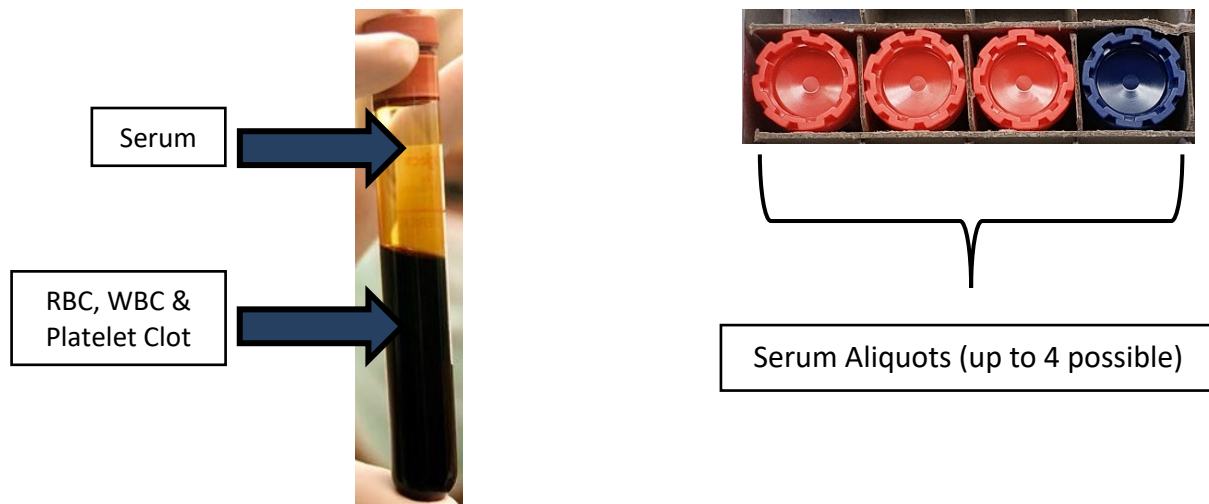
6.4 Whole Blood Collection with 10 ml Serum (Red-Top) Tube for Serum

1. Set centrifuge 4°C to pre-chill before use.
2. Place completed HEAD ID Label and **SERUM** Collection Tube Label on the Plain Red-Top Serum Blood Collection Tube. Place pre-printed **SERUM** Cryovial Labels on the three 2 ml cryovials with red caps and one 2 ml cryovial with blue cap (if necessary, for residual).
3. Using a blood collection set and a holder, collect blood into **Plain Red-Top Serum Blood Collection Tubes (10 ml)** using your institution's recommended procedure for standard venipuncture technique

The following techniques shall be used to prevent possible backflow:

- a. Place participant's arm in a downward position.
 - b. Hold tube in a vertical position, below the participant's arm during blood collection.
 - c. Release tourniquet as soon as blood starts to flow into tube.
 - d. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into each tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
 - a. If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional Serum tube at this time. Process blood obtained in existing Serum tube.
 5. Immediately after blood collection, gently invert/mix (180 degree turns) each tube 5 times.
 6. 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 a placing the pipette tip along the lower side of the wall without agitating the packed red blood cells at the bottom of the collection tube.
 9. Transfer serum into the pre-labeled cryovials with red caps. The serum tube should yield, on average, 4-5 ml of serum. Aliquot 1.5 ml serum into each cryovial. Be sure to only place **serum** in cryovials with red caps and labeled with **SERUM** labels. Place residual serum (<1.5 ml) in the blue-capped cryovial. **If a residual aliquot (<1.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**

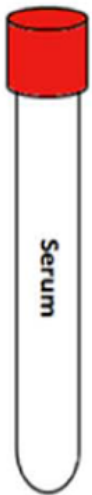


10. Place the labeled cryovials in the 48 cell cryobox and place on dry ice. Transfer to -80°C Freezer when possible. Store all samples at -80°C until shipped to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

Serum Preparation (10ml Red Top Tube)



Step One



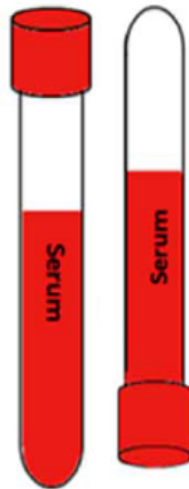
- Store tubes at room temperature.
- Label tubes and cryovials with pre-printed subject labels prior to blood draw.

Step Two



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

Step Three



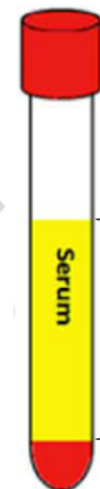
- Immediately after blood draw, invert tube 5 times to mix samples.

Step Four

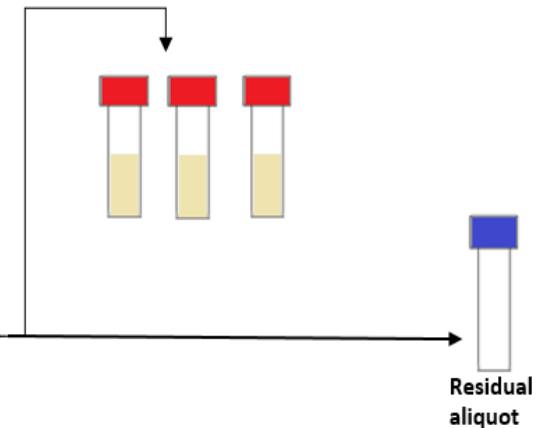


- Allow blood to clot for 30 minutes.
- Within 60 minutes of blood draw, centrifuge samples at 2000 x g for 10 minutes at 4°C.

Step Five



- Must be spun, aliquoted, and stored in -80°C freezer within 2 hours of collection.



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

6.5 EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Plasma and Buffy Coat x5

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 HEAD ID Label and pre-printed “**PLASMA**” Collection Tube Label on the lavender-top EDTA tubes. Place pre-printed “**PLASMA**” Cryovial Labels on the (16) 2.0 ml cryovials with purple caps and (1) 2.0 ml cryovial with blue cap (if necessary, for residual). Place pre-printed “**BUFFY COAT**” Cryovial Label on the (5) 2.0 ml cryovials with gray caps.
3. Using a blood collection set and a holder, collect blood into the **EDTA (Lavender-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 participant's arm in a downward position.
 - b. Hold tube in a vertical position, below the participant's arm during blood collection.
 - c. Release tourniquet as soon as blood starts to flow into tube.
 - d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.

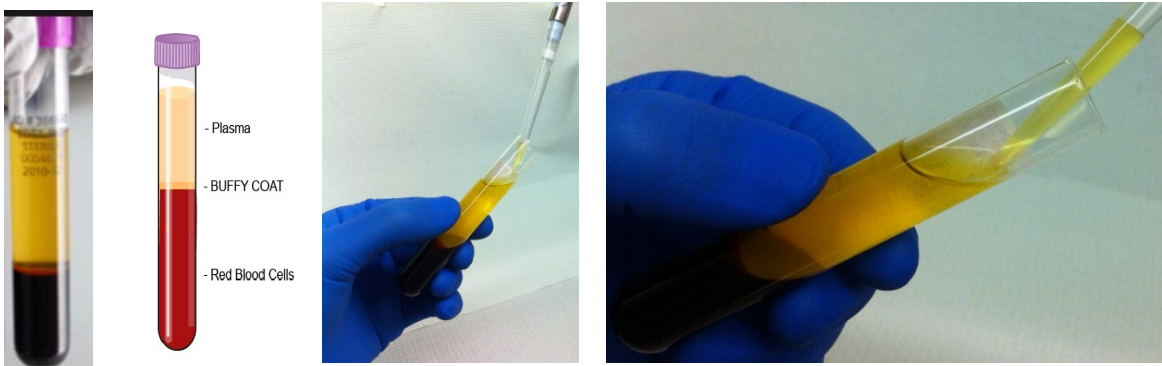
If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.

5. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tubes 8-10 times.**
6. **CRITICAL STEP: Immediately after inverting the EDTA tubes, place them on wet ice until centrifugation begins.**

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

8. Remove the plasma, being careful not to agitate the packed red blood cells at the bottom of the tube. Tilt the tube and place a disposable pipette tip along the lower side of the wall without touching the pellet (buffy coat) so that plasma is not contaminated (see below). Transfer plasma from all five EDTA tubes into the 50 ml conical tube and gently invert 3 times.

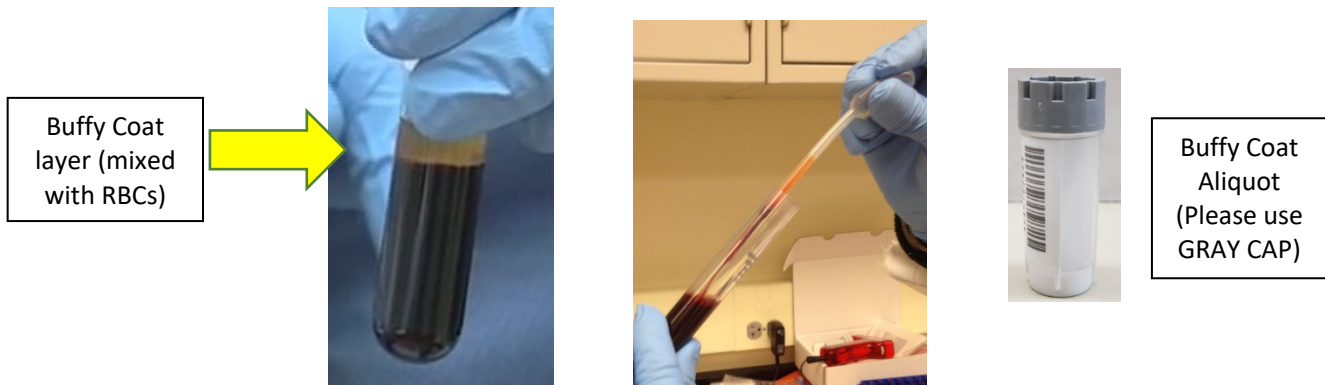
9. Aliquot 1.5 ml per cryovial (total vials = up to 16 with 1.5 ml each). Each EDTA tube should yield, on average, 4-5 ml of plasma. Be sure to only place **plasma** in cryovials with purple caps and labeled with “PLASMA” labels. Take caution not to disturb the red blood cells at the bottom of the tube. If there is extra plasma left, use 1 blue-capped cryovial with “PLASMA” label 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.

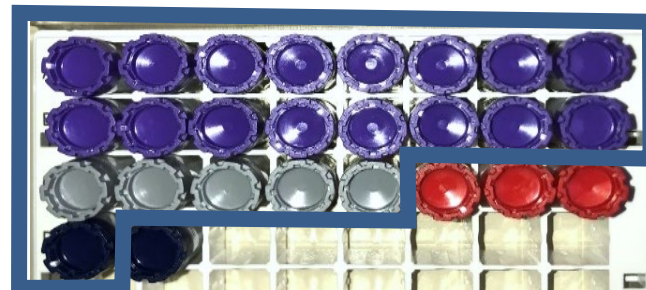
10. Place the labeled cryovials in the 48-slot cryovial box and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample Shipment and Notification Form.

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



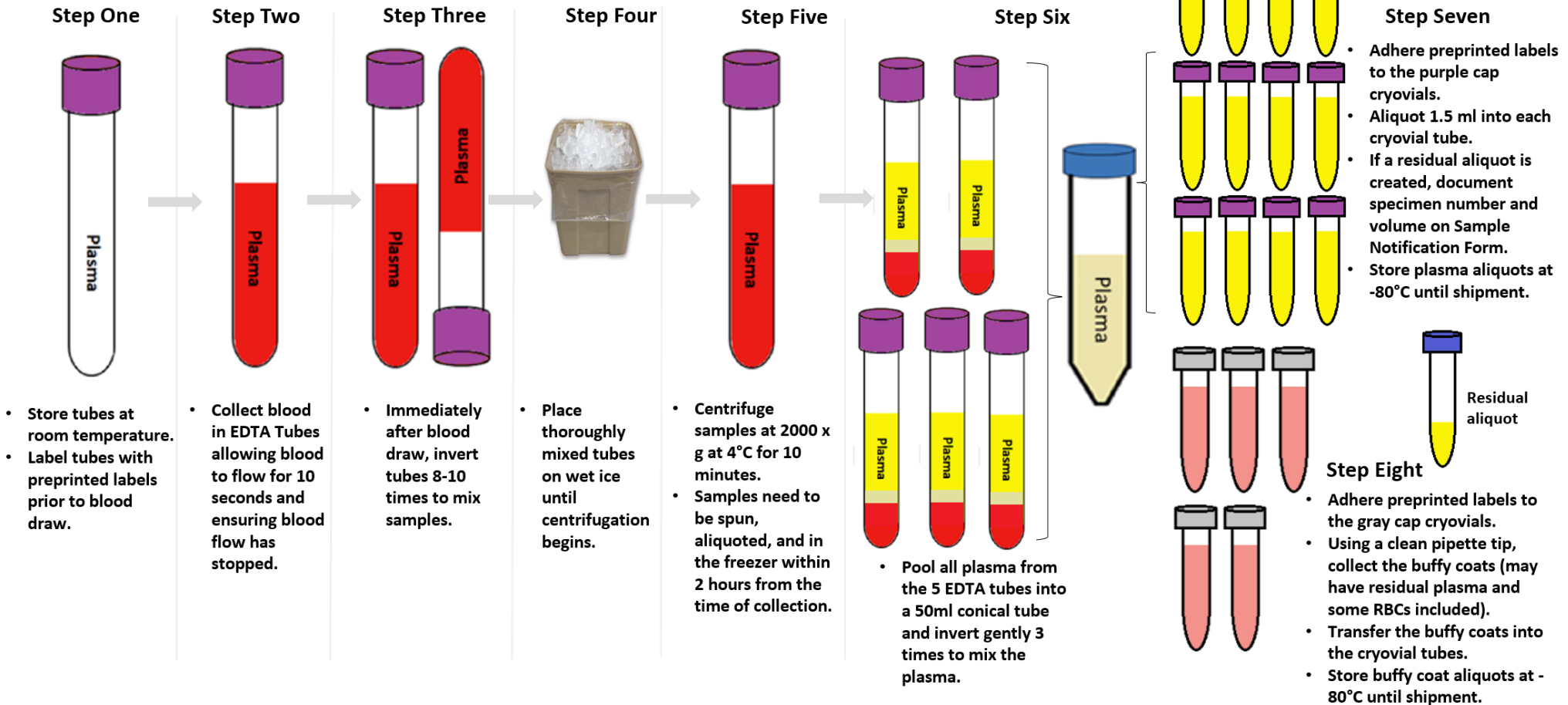
12. Dispose of tube with red blood cell pellet according to your site’s guidelines for disposing of biomedical waste.

13. Place the labeled cryovials in the 48-slot cryovial box and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice



Plasma Aliquots (up to 17 possible) and Buffy Coat (5)

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



6.6 EDTA (Lavender-Top) Blood Collection Tube (6ml)



1. Place completed PT ID Label and pre-printed “**WBLD**” Collection Tube Label on the 6ml lavender-top EDTA tube.
2. Using a blood collection set and a holder, collect whole blood into the 6 ml lavender top whole blood tube using your institution’s recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

- a. Place participant's arm in a downward position.
 - b. Hold tube in a vertical position, below the participant’s arm during blood collection.
 - c. Release tourniquet as soon as blood starts to flow into tube.
 - d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
3. Invert the tube gently 3 times.
 4. Transfer the tube immediately to a **-80°C Freezer**. The sample should be frozen and stored **UPRIGHT** in a WIRE or PLASTIC type test tube rack (DO NOT use a solid Styrofoam test tube holder).



Whole Blood Preparation (6 mL Lavender-Top Tube)



Step One



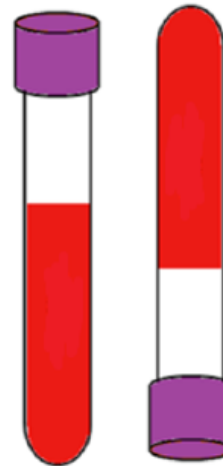
- Store tubes at room temperature.
- Label tubes with pre-printed subject labels prior to blood draw.

Step Two



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

Step Three



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

Step Four



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



7.0 INCOMPLETE OR DIFFICULT BLOOD DRAWS

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

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

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

Draw Tube Order	Sample Type	Tube Type	Tubes to NCRAD	Ship
1	Whole blood for transcriptome analysis	PAXgene™ Blood RNA Collection Tube (2.5 ml) for RNA	1	Frozen
2	Whole blood for isolation for serum	SERUM: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	Up to 4	Frozen
3	Whole blood for isolation of plasma & buffy coat (for DNA extraction)	PLASMA: 2.0 ml cryovials with purple cap (residual volume placed in 2.0 ml cryovial with blue cap)	Up to 17	Frozen
		BUFFY COAT: 2.0 ml cryovial	5	Frozen
4	Whole blood for future analysis	EDTA (Lavender-Top) Blood Collection Tube (6 ml)	1	Frozen

8.0 PACKAGING AND SHIPPING INSTRUCTIONS

ALL study personnel responsible for shipping should be certified in biofluid shipping (i.e. IATA certification). If not available at your institution, please contact NCRAD with questions and information regarding resources.

8.1 Frozen Packaging Information

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

IMPORTANT!

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

Specimens being shipped to NCRAD should be considered as Category B UN3373 specimens and as such must be triple 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

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

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

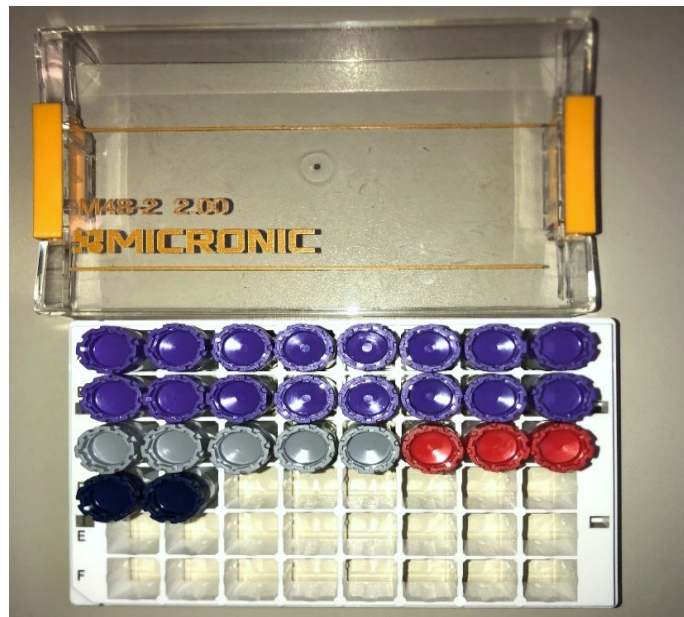


rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning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

8.1.1 Frozen Packaging Instructions

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

- a. Completed Biological Sample and Shipment Notification Form to the email notification. (See [Appendix B](#) for an example of the NCRAD sample form)
 - b. If email is unavailable, please call NCRAD (800-526-2839) and do not ship until you have contacted and notified NCRAD coordinators about the shipment in advance.
2. Place all frozen labeled aliquots of plasma and buffy coat aliquots from the same subject in the cryovial cryobox.
 - a. Each 48-slot cryobox will hold approximately 26 cryovial samples. Place serum, plasma and buffy coat within one cryobox (4 serum, 17 plasma, 5 buffy coat) per participant blood draw (see below).



One cryobox containing serum, plasma, residuals, and buffy coat aliquots.

- b. Cryoboxes should contain all of the specimens from the same patient, per time point.
 - c. **Batch shipping should be performed every (3) three months or when specimens from 5 participants accumulates, whichever is sooner.**
3. Label the outside of the cryoboxes with the appropriate kit number label(s). Place serum, plasma and buffy coat aliquots within one cryobox and place within a biohazard bag. The biohazard bags are large

enough to contain one cryobox and 1 frozen blood tube from one subject's visit.

4. Place the cryobox in the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag). Place frozen 6ml EDTA tube and 2.5ml PAXgene™ tube in provided bubble wrap tube sleeves, seal and place in biohazard bag. Seal biohazard bag according to the instructions on the bag.



Place kit number label(s) on cryobox

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

Full Shipping Container with
Batched Samples and Dry Ice



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

IMPORTANT!

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

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

HEAD at NCRAD
Indiana University School of Medicine
351 West 10th Street
TK-217

Indianapolis, IN 46202
Phone: 1-800-526-2839

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

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

For frozen shipments, include no more than five cryovial boxes (separated by patient within 5 biohazard bags) per shipping container in order to have room for a sufficient amount of dry ice to keep samples frozen up to 24 hours.

The labeled, processed, aliquoted, and frozen cryovials of serum, plasma and buffy coat will be shipped to NCRAD as outlined above.

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

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

8.2 Frozen Shipping Instructions

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



Shipping ▾

History

End of Day ▾

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

Ship From

Company	
Contact	
Address 1	
Address 2	
Address 3	
City	
State/Province	
Postal Code	
Country/Territory	▼
Phone	

4. Click on the magnifying glass icon in the “Ship From” section to search for your shipping address.
 - a. Search by Company (site), Contact (name), or Address 1 (first line of your site’s street address). Click Search.
 - b. Click Select to the left of the correct contact information.
5. Verify that both the shipping information AND study reference are correct for this shipment.
 - a. If wrong study contact or study reference, click Reset in the bottom right of the screen to research for the correct information.
6. Enter Package Information
 - a. Ambient shipments
 - i. Enter the total weight of your package in the “Weight” field and leave the “Dry Ice Weight” field empty.
 - b. Frozen shipments
 - i. Enter the total weight of your package in the “Weight” field.
 - ii. Enter the dry ice weight in the “Dry Ice Weight” field.
 - iii. If the “Dry Ice Weight” field is higher than the “Weight” field, you will receive an error message after clicking “Ship” and need to reenter these values.

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

9.0 DATA QUERIES AND SAMPLE RECONCILIATION

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

10.0 APPENDICES LIST

[Appendix A: Rate of Centrifugation Worksheet](#)

[Appendix B: Biological Sample and Shipment Notification Form](#)

Appendix A Rate of Centrifuge Worksheet

Please complete and return this form by email to the NCRAD Study Team 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:

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

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 at alzstudy@iu.edu

Appendix B: Biological Sample and Shipment Notification Form

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

To: Diont'e Keys Email: alzstudy@iu.edu Phone: 1-800-526-2839

General Information:

From: _____
 Phone: _____

Tracking #: _____
 Date: _____
 Email: _____

Study: HEAD **Visit (circle one):** BASELINE 18M

Site ID: _____ **PTID:** _____

Kit #: _____ **KIT BARCODE**

Sex (circle one): M F **Year of Birth:** _____

Blood Collection:

Date Drawn: _____ [MMDDYY]	Time of Draw: _____ [HHMM]
Last date subject ate: _____ [MMDDYY]	Last time subject ate: _____ [HHMM]

Lavender Top Whole Blood Tube (6 ml)				RNA (PAXgene™ Tube)			
Original volume drawn: _____ mL	_____ mL	Time EDTA tube placed in freezer: _____ [HHMM]	_____ [HHMM]	Original volume drawn: _____ ml	_____ ml	Time PAXgene™ tube placed in freezer: _____ [HHMM]	_____ [HHMM]

Blood Processing:

Serum (Red-top) Tube (10 mL)			
Time spin started: _____ [HHMM]	_____ [HHMM]	Number of 1.5 mL serum aliquots created (red cap): _____	_____
Duration of centrifuge: _____ Minutes	_____ Minutes	If applicable, volume of residual serum aliquot (less than 1.5 mL in blue cap): _____ mL	_____ mL
Temp of Centrifuge: _____ °C	Rate of centrifuge: _____ x g	If applicable, specimen number of residual serum aliquot (last four digits): _____	_____
Original volume drawn: _____ mL	_____ mL	Time aliquots placed in freezer: _____ [HHMM]	_____ [HHMM]
Time aliquoted: _____ [HHMM]	_____ [HHMM]	Storage temperature in freezer: _____ °C	_____ °C

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

Notes: