



## ABC-DS Manual of Procedures Update

**V05.2023**

Section	Change
Document Footer	The version date was updated for this amendment.
Title Page	Title page updated to reflect version change.
Table of Contents	Updated page numbers.
Section 5.1	Order of tubes in kit contents rearranged to reflect draw order.
Section 5.2	Updated kit order return timeline to three weeks due to supply chain issues.
Section 6.1	<ol style="list-style-type: none"> <li>1. Updated pictures of the Collection and Aliquot Labels that now have 2 barcodes.</li> <li>2. Updated instructional labeling pictures to show cryovial ridges.</li> </ol>
Section 6.3	If unable to create full aliquots to send to UNTHSC, make note of low-volume samples on Appendix F.
Section 6.4	Updated color of Serum in schematic for consistency.
Section 6.5	Added picture of checkbox on Appendix B to make directions clearer.
Section 6.6	Updated color of Plasma in schematic for consistency.
Section 8.0	Updated table to reflect draw order.
Section 8.1	Moved step 14 to step 2 to make direction clearer.
Section 12.1	Order of tubes in kit contents rearranged to reflect draw order.
Section 13.1	<ol style="list-style-type: none"> <li>1. Updated pictures of the Collection and Aliquot Labels that now have 2 barcodes.</li> <li>2. Updated instructional labeling pictures to show cryovial ridges.</li> </ol>
Section 13.3	Added link to Appendix F for documenting residual aliquots.
Section 13.4	<ol style="list-style-type: none"> <li>1. Updated color of Serum in schematic for consistency.</li> <li>2. Corrected directions for documenting residual aliquots.</li> </ol>
Section 13.5	<ol style="list-style-type: none"> <li>1. Updated color of Plasma in schematic for consistency.</li> <li>2. Corrected directions for documenting residual aliquots.</li> </ol>
Section 14.1	Moved step 14 to step 2 to make direction clearer.
Throughout Document	Removal of Serum Discard Tube due to recent confirmation that Serum Discard Tube is not required to be drawn prior to drawing RNA PAXgene™ tube.
Addendum 1	<p>MOM'S Substudy Addendum</p> <ol style="list-style-type: none"> <li>1. Updated sample label pictures to reflect MOM's Substudy protocol.</li> <li>2. Updated picture of cryobox lid to contain "MOM" label to help differentiate main study samples from Substudy Samples upon intake at UNTHSC.</li> </ol>



## **Alzheimer's Biomarker Consortium – Down Syndrome**

in collaboration with

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

*Blood-Based Biospecimens*

*Manual of Procedures*

*Version 3.2*

*May 2023*



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## 1.0 ABBREVIATIONS

ABC-DS	Alzheimer's Biomarker Consortium – Down Syndrome
AD	Alzheimer's Disease
APOE	Apolipoprotein E
ATRI	Alzheimer's Therapeutic Research Institute
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
DS	Down Syndrome
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
IU Path Lab	Indiana University Pathology Laboratory
LP	Lumbar Puncture
miRNA	Micro Ribonucleic Acid
NaHep	Sodium Heparin (Green-Top) Blood Collection Tube (4 mL)
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
RBC	Red Blood Cells
RNA	Ribonucleic Acid
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
SST	Serum Separator Tube
UNTHSC	University of North Texas – Health Science Center

## 2.0 PURPOSE

The collection of blood-based biofluids is an important part of the Alzheimer's Biomarker Consortium – Down Syndrome (ABC-DS) study. These samples will be used to:

- Identify critical factors important to neurodegeneration and, ultimately, dementia
- Define biomarkers for the above factors
- Set a foundation for an efficient transition from this biomarker study to a therapeutic trial to combat AD in DS augmented by biomarker outcomes

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 blood-based biological samples for ABC-DS study visits.

*The following samples will be sent to NCRAD:*

- Serum
- Sodium Heparin (NaHep) – (karyotyping)
- Plasma
- Buffy Coat – (DNA extraction)
- RNA – (miRNA extraction)

*Additional samples collected but not shipped to NCRAD:*

- Serum – shipped to UNTHSC
- Plasma – shipped to UNTHSC
- CSF – shipped to Washington University – St. Louis (Please see the [ABC-DS Lumbar Puncture Manual of Procedures for details](#))
- Clinical Labs – shipped to IU Path Lab
  - Serum 5.0mL orange-top x1
  - Serum 5.0mL gold-top x1
  - EDTA 3.0mL x1

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

These procedures are relevant to all study personnel responsible for processing blood specimens entering UNTHSC, NCRAD and IU Path Lab.



### 3.0 **CONTACT INFORMATION**

#### 3.1 **NCRAD Contacts**

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**Zoë Potter, BA, CCRP, Study Coordinator**

Phone: (317) 278-9086

Email: [zdpotter@iu.edu](mailto:zdpotter@iu.edu)

#### **General NCRAD Contact Information**

Phone: 1-800-526-2839

Email: [alzstudy@iu.edu](mailto:alzstudy@iu.edu)

Website: [www.ncrad.org](http://www.ncrad.org)

ABC-DS Study Specific Webpage:

[NCRAD - The ABC-DS Active Study Page](#)

#### 3.2 **Sample Shipment Mailing Addresses:**

##### **NCRAD**

Indiana University School of  
Medicine

351 West 10<sup>th</sup> Street

TK-217

Indianapolis, IN 46202

[alzstudy@iu.edu](mailto:alzstudy@iu.edu)

##### **UNTHSC**

3420 Darcy Street

Fort Worth, TX 76107

Phone: 817-735-2638

[Tori.Como@unthsc.edu](mailto:Tori.Como@unthsc.edu)

##### **IU Path Lab**

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### 3.3 Hours of Operation

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

**UNTHSC** business hours are from 8 AM to 5 PM **Central Time**, Monday through Friday.

Frozen samples must be shipped **Monday-Wednesday only** from US sites and **Monday only** from Cambridge. For packaging and shipment details of frozen samples, please refer to [Section 8.1](#) of this protocol.

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

### 3.4 NCRAD Holiday Observations

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

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

- 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.
- For holiday observations regarding shipments to UNTHSC, please follow the guidelines as outlined above for Indiana University.
- **Please see:** [https://ncrad.org/holiday\\_closures.html](https://ncrad.org/holiday_closures.html) for additional information.





## 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 (21 gauge) and hub
- Microcentrifuge tube rack
- Sharps bin and lid
- Wet Ice Bucket

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:

- For NCRAD/UNTHSC: Centrifuge capable of  $\geq 2000 \times g$  with refrigeration to 4°C
- For IU Path Lab: Centrifuge capable of 1300 x 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)

### 4.2 Biospecimens Collection Schedules

#### **ABC-DS Blood-Based Biomarker Collection Schedule for DS Participants and Sibling Controls:**

	Serum	Plasma	DNA	RNA	Karyotyping <sub>1</sub>
All visits	X	X	X	X	X
SHIP TO:	NCRAD & UNTHSC	NCRAD & UNTHSC	NCRAD	NCRAD	NCRAD

<sub>1</sub>DS Participants only (if needed)

**Clinical Labs Blood Collection Schedule for DS Participants ONLY**

	<b>Serum – Free T4, Thyroid, Triiodothyronine, TSH, Vit B12, ATA Preparation</b>	<b>Vit D, BMP, Lytes, Lipid Preparation</b>	<b>CBC Preparation</b>	<b>A1C Preparation</b>
Cycle 1	X	X	X	X
Cycle 2	X	X	X	X
SHIP TO:	IU PATH LAB	IU PATH LAB	IU PATH LAB	IU PATH LAB

Whole blood is collected for the main study protocol (NCRAD and UNTHSC) in up to four different types of tubes (2) 5mL gold-top serum separator tube, 4mL green-top Sodium Heparin (NaHep) tube, (2) 10mL lavender-top EDTA tube and a 2.5 mL PAXgene™ tube. Whole blood is collected for the Clinical Labs (IU Path Lab) in up to three different types of tubes (1) 5 mL orange-top serum separator tube, (1) 5mL gold-top serum separator tube and (1) 3mL lavender-top EDTA tube.

NCRAD and UNTHSC

- The 10mL EDTA is processed locally into plasma and buffy coat fractions. They are then aliquoted, frozen at the study site and shipped to NCRAD and UNTHSC.
- The (2) 5mL gold top serum separator tubes are processed locally into serum fractions. They are then aliquoted, frozen at the study site and shipped to NCRAD and UNTHSC.
- The Sodium Heparin tubes are shipped to NCRAD on the day of the participant visit if karyotyping is needed (Monday through Thursday only).
- The PAXgene™ tube is frozen locally without further processing and shipped to NCRAD.

IU Path Lab-Clinical Labs

- The 5mL orange-top serum tube for Free T4, Thyroid, Triiodothyronine, TSH, Vit B12, ATA Preparation is processed locally into serum and shipped refrigerated to IU Path Lab on the day of the participant visit.
- The 5mL gold-top serum tube for Vit D, BMP, Lytes and Lipid Preparation is processed locally into serum and shipped refrigerated to IU Path Lab on the day of the participant visit.
- The 3mL lavender-top EDTA tube of whole blood for A1C and CBC Preparation is shipped refrigerated to IU Path Lab on the day of the participant visit.

*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.*

*If consent to bank with NCRAD is not obtained, only draw 1 EDTA (Lavender-Top) Blood Collection Tube (10 mL) and 1 Serum Separator (Gold-Top) Blood Collection Tube (5 mL). Send 2 serum aliquots and 17 plasma aliquots to UNTHSC. If consent for genetic research is not obtained, do not send buffy coats to NCRAD, since buffy coats are the source of DNA for said genetic research.*

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 following tables.

#### **4.2.1 Re-Draw Instructions and Timeframes**

Sample Collection-Blood eCRF is a log form. Select 'Add a new record' to enter a record. Enter one record per Date of Collection and specify samples collected. At least one sample type must be marked as collected on this date to successfully submit the form.

If a re-draw is necessary and occurs BETWEEN TWO VISITS, add a new record in the visit PRIOR to the re-draw timeframe, making sure to include the re-draw date of collection and Kit Number. If a sample was missed during a regularly scheduled visit, but a sample was collected PRIOR to NEXT scheduled visit, enter in the EDC as a re-draw. Also, provide reason for re-draw in the comments section.

For ABC-DS, the re-draw timeframe is as follows:

- For all visits, the re-draw timeframe will be up to 3 months prior and 3 months after the expected visit date.

**4.3 Biospecimen Collection Chart**  
**Research Blood Collection for DS Participants and Sibling Controls**

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Tubes to UNTHSC	Ship
Whole blood for isolation of serum	Serum Separator (Gold-Top) Blood Collection Tube (5 mL)	2	N/A	N/A	N/A	N/A
	SERUM: 0.5 mL cryovials	21	0.25 mL serum aliquot per 0.5 mL Cryovial (clear cap)	19	2	Frozen
Whole blood for Karyotyping*	Sodium Heparin (Green-Top) Blood Collection tube (4 mL)	1	N/A	1	0	Ambient
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	EDTA (Lavender-Top) Blood Collection Tube (10 mL)	2	N/A	N/A	N/A	N/A
	PLASMA: 0.5 mL cryovials	41	0.25 mL plasma aliquot per 0.5 mL cryovial (clear cap)	24	17	Frozen
	BUFFY COAT: 2.0 mL cryovials	2	1 mL buffy coat aliquot per 2.0 mL cryovial (BLUE CAP)	2	0	Frozen
Whole blood for RNA extraction	PAXgene™ Blood Collection Tube (2.5 mL)	1	N/A	1	0	Frozen

\*DS participants only

#### 4.3.1 Clinical Labs Blood Collection for DS Participants ONLY

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to IU Path Lab	Ship
Whole blood for isolation of serum	Serum Separator (Orange-Top) Blood Collection Tube (5 mL)	1	N/A	N/A	N/A
	SERUM: 2.0 mL cryovials	2	1.0 mL serum aliquot per 2.0mL cryovial (CLEAR CAP)	2	Refrigerated
	Serum Separator (Gold-Top) Blood Collection Tube (5 mL)	1	N/A	N/A	N/A
	SERUM: 2.0 mL cryovials	2	1.0 mL serum aliquot per 2.0mL cryovial (RED CAP)	2	Refrigerated
Whole Blood for CBC Preparation	EDTA (Lavender-Top) Blood Collection Tube (3 mL)	1	N/A	1	Refrigerated
Whole Blood for A1C Preparation					

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the **IU Path Lab form** ([Appendix D](#)). Submit a copy to IU Path Lab with a reason provided for the omission.

## 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; 2) CSF collection kits including Lumbar Puncture (LP) trays, the CSF Supplemental Supply Kit and the CSF Shipping Supply Kit; and 3) clinical lab supplies (with the exception of dry ice and equipment supplies listed in [Section 4.1](#)). These materials include blood tubes, pipettes, pipette tips, LP trays (when applicable), boxes for serum/plasma/buffy coat/CSF aliquots, as well as partially completed shipping labels to send materials to UNTHSC, NCRAD and the IU Path Lab. Kit Number Labels, Site and BDS ID Labels, Collection and Aliquot Tube Labels will all be provided by NCRAD. Details regarding the CSF Kits are found in the [ABC-DS LP Manual of Procedures](#). Collection and Aliquot Tube Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to [Section 6.1](#).

### 5.1 Specimen Collection Kit Contents

Collection kits contain the following (for each 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.

#### ABC-DS Blood-Based Kit for DS Participants

Quantity	ABC-DS DS Participant Blood Kit
2	Serum Separator (Gold-Top) Blood Collection Tube (5 mL)
2	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
1	PAXgene™ Blood Collection Tube (2.5 mL)
2	15 mL conical
21	Cryovial tube (0.5 mL) with clear cap (Serum)
41	Cryovial tube (0.5 mL) with clear cap (Plasma)
2	Cryovial tube (2.0 mL) with blue cap
3	Disposable graduated transfer pipette (1 mL)
69	Pre-printed Collection and Aliquot Tube Label
5	Pre-printed Kit Number Label
5	Labels for handwritten Site and BDS ID
1	3"x5" self-seal bubble bag
1	Resealable bag
1	Cryobox 81-cell
1	Microcentrifuge tube box (holds up to 25 microcryovials)

**ABC-DS Blood-Based Kit for Sibling Controls**

<b>Quantity</b>	<b>ABC-DS Sibling Control Blood Kit</b>
2	Serum Separator (Gold-Top) Blood Collection Tube (5 mL)
2	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
1	PAXgene™ Blood Collection Tube (2.5 mL)
2	15 mL conical
21	Cryovial tube (0.5 mL) with clear cap (Serum)
41	Cryovial tube (0.5 mL) with clear cap (Plasma)
2	Cryovial tube (2.0 mL) with blue cap
3	Disposable graduated transfer pipette (1 mL)
69	Pre-printed Collection and Aliquot Tube Label
5	Pre-printed Kit Number Label
5	Labels for handwritten Site and BDS ID
1	3"x5" self-seal bubble bag
1	Resealable bag
1	Cryobox 81-cell
1	Microcentrifuge tube box (holds up to 25 microcryovials)

**ABC-DS Blood-Based Kit for Clinical Labs**

<b>Quantity</b>	<b>ABC-DS Blood Kit for Clinical Labs</b>
1	Serum Separator (Orange-Top) Blood Collection Tube (5 mL)
1	Serum Separator (Gold-Top) Blood Collection Tube (5 mL)
1	EDTA (Lavender-Top) Blood Collection Tube (3 mL)
2	Cryovial tube (2.0 mL) with clear cap
2	Cryovial tube (2.0 mL) with red cap
2	Disposable graduated transfer pipette (3mL)
8	Labels for handwritten Site BDS ID and DOB
1	Resealable bag
1	Microcentrifuge tube box (holds up to 25 microcryovials)
3	Resealable tube pouches, on roll
1	Pre-printed UPS Return Airbill addressed to IU Path Lab
1	Biohazard bags with absorbent sheet (small)
1	X-Small insulated shipper
2	Cold pack
1	Brown corrugated box
5	Air cushions
1	Warning label packet
1	Round fluorescent "ABC-DS Study" sticker
1	Large rectangular fluorescent "ABC-DS Study" sticker

**ABC-DS Frozen Blood Shipping Supply Kit**

Quantity	ABC-DS Frozen Blood Shipping Kit
5	Plastic Biohazard bag with absorbent sheet (large)
5	Biohazard bag with Absorbent sheet (small)
2	Shipping airbill
2	Resealable bag
2	Shipping box/Styrofoam container
2	Warning label packet with dry ice sticker

\*Cambridge uses their own shipping containers, airbills, and warning labels.

**ABC-DS Ambient Blood Shipping Supply Kit**

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

**ABC-DS Blood Supplemental Supply Kit**

Quantity	Blood Supplemental Supply Kit
10	Serum Separator (Gold-Top) Blood Collection Tube (5 mL)
5	Serum Separator (Orange-Top) Blood Collection Tube (5 mL)
10	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
5	EDTA (Lavender-Top) Blood Collection Tube (3 mL)
5	PAXgene™ Blood Collection Tube (2.5 mL)
10	15 mL conical
25	Cryovial tube (0.5 mL) with clear cap (Serum)
50	Cryovial tube (0.5 mL) with clear cap (Plasma)
10	Cryovial tube (2.0 mL) with blue cap
15	Disposable graduated transfer pipette (3 mL)
10	Labels for handwritten Site and BDS ID
10	3"x5" self-seal bubble bag
10	Resealable bag
5	Cryobox 81-cell
5	Microcentrifuge tube box (holds up to 25 microcryovials)
5	Plastic Biohazard bag with absorbent sheet (large)
5	Biohazard bag with Absorbent sheet (small)



5	FedEx return airbill and pouch
2	Shipping box/Styrofoam container
2	Warning label packet with dry ice sticker

**Individual Supplies**

<b>Quantities</b>	<b>Items Available upon request within the NCRAD kit module.</b>
By Request	Serum Separator (Gold-Top) Blood Collection Tube (5 mL)
By Request	Serum Separator (Orange-Top) Blood Collection Tube (5 mL)
By Request	Sodium Heparin (Green-Top) Blood Collection Tube (4 mL)
By Request	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
By Request	EDTA (Lavender-Top) Blood Collection Tube (3 mL)
By Request	PAXgene™ Blood Collection Tube (2.5 mL)
By Request	15 mL conical
By Request	Cryovial tube (0.5 mL) with clear cap
By Request	Cryovial tube (2.0 mL) with blue cap
By Request	Disposable graduated transfer pipette
By Request	Labels for handwritten Site and BDS ID
By Request	3"x5" self-seal bubble bag
By Request	Resealable bag
By Request	Cryobox 81-cell
By Request	Microcentrifuge tube box (holds up to 25 microcryovials)
By Request	Plastic Biohazard bag with absorbent sheet (large)
By Request	Biohazard bag with Absorbent sheet (small)
By Request	FedEx return airbill
By Request	Resealable bag
By Request	Shipping box/Styrofoam container
By Request	Warning label packet with dry ice sticker
By Request	Small IATA shipping box with insulated cooler
By Request	Small refrigerant pack
By Request	Aqui-Pak 6 tube absorbent pouch
By Request	UN3373 Biological Substance Category B label
By Request	List of contents card
By Request	Shipping Clin Pak

## 5.2 Kit Supply to Study Sites

Each individual site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits. Please go to [ABC-DS Kit Request System](#) 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 **THREE weeks** for kit orders to be processed and delivered.

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

## 6.0 BLOOD COLLECTION AND PROCESSING PROCEDURES

### \*\*\*Important Note\*\*\*

In order to ensure the highest quality samples are collected, processed, and stored, it is essential to follow the specific collection, processing, and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood. There are 2 options for blood draw order:

#### Draw Order – Option 1 (PREFERRED):

##### Research collection (Day 1):

- 1. Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 2
- 2. Sodium Heparin (Green-Top) Blood Collection Tube (4 mL) for Karyotyping (DS Participants only, as needed)
- 3. EDTA (Lavender-Top) Blood Collection Tube (10 mL) for DNA and Plasma x 2
- 4. PAXgene™ Blood Collection Tube (2.5 mL) for RNA

##### Clinical labs collection (Day 2):

- ❖ 1. Serum Separator (Orange-Top) Blood Collection Tube (5 mL) for Serum x 1
- ❖ 2. Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 1
- ❖ 3. EDTA (Lavender-Top) Blood Collection Tube (3mL) for hematology

#### Draw Order – Option 2:

##### Collection – Research and Clinical Labs on same day/visit:

- 1. Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 2 (NCRAD)
- ❖ 2. Serum Separator (Orange-Top) Blood Collection Tube (5 mL) for Serum x 1 (IU Path Lab)
- ❖ 3. Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 1 (IU Path Lab)
- 4. Sodium Heparin (Green-Top) Blood Collection Tube (4 mL) for Karyotyping (DS Participants only, as needed) (NCRAD)
- 5. EDTA (Lavender-Top) Blood Collection Tube (10 mL) for DNA and Plasma x 2 (NCRAD)
- ❖ 6. EDTA (Lavender-Top) Blood Collection Tube (3 mL) for hematology (IU Path Lab)
- 7. PAXgene™ Blood Collection Tube (2.5 mL) for RNA x 1 (NCRAD)

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

## 6.1 Labeling Samples

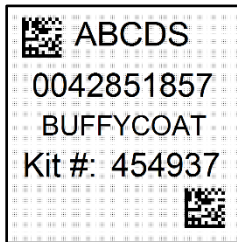
**\*\*Label Type Summary\*\***

1. Kit Number Label
2. Collection and Aliquot Tube Label
3. Site and BDS ID Label
4. Site BDS ID and DOB Labels

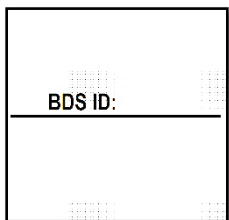
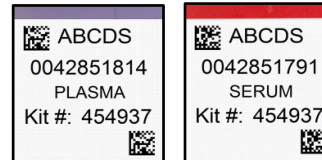


The **Kit Number Labels** do not indicate a specimen type but are affixed on the Biological Sample and Shipment Notification Forms and on specific packing materials for UNTHSC and NCRAD.

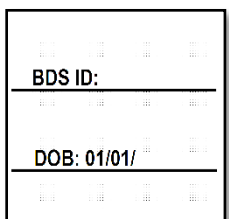
**\*\* Extra Kit Number Label included in each Blood kit in case NaHep tube requested for karyotyping.**



The **Collection and Aliquot Tube Labels** for blood derivatives and CSF are placed on all collection and aliquot tubes for UNTHSC and NCRAD. **Note:** *Aliquot Tube Labels for Plasma and Serum are color-coded to replace cap stickers.*



The **Site and BDS ID Labels** are placed on all blood collection tubes for UNTHSC and NCRAD.

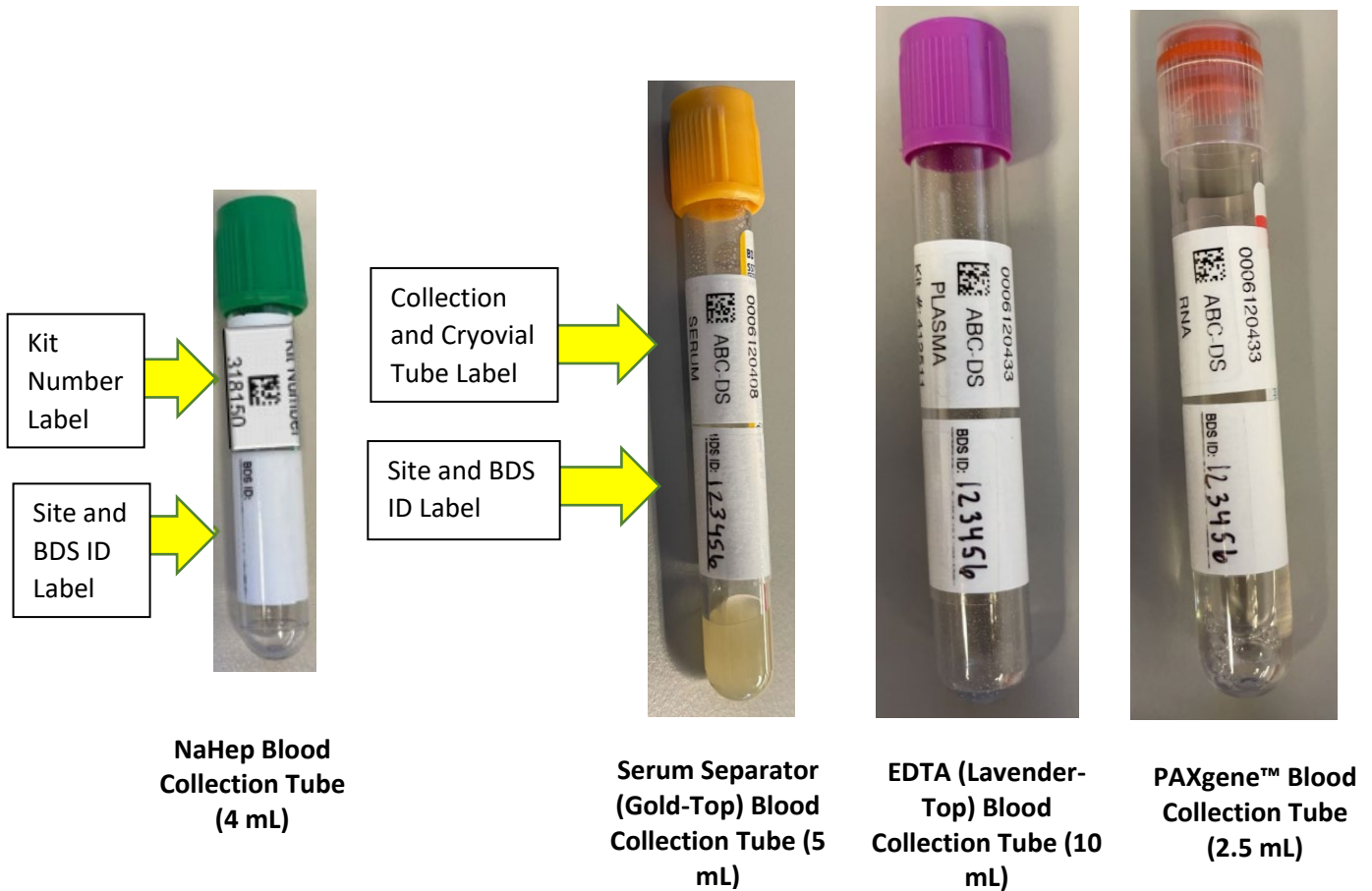


The **Site BDS ID and DOB Labels** are placed on all tubes for IU Path Lab.

**\*\*Important Note\*\***

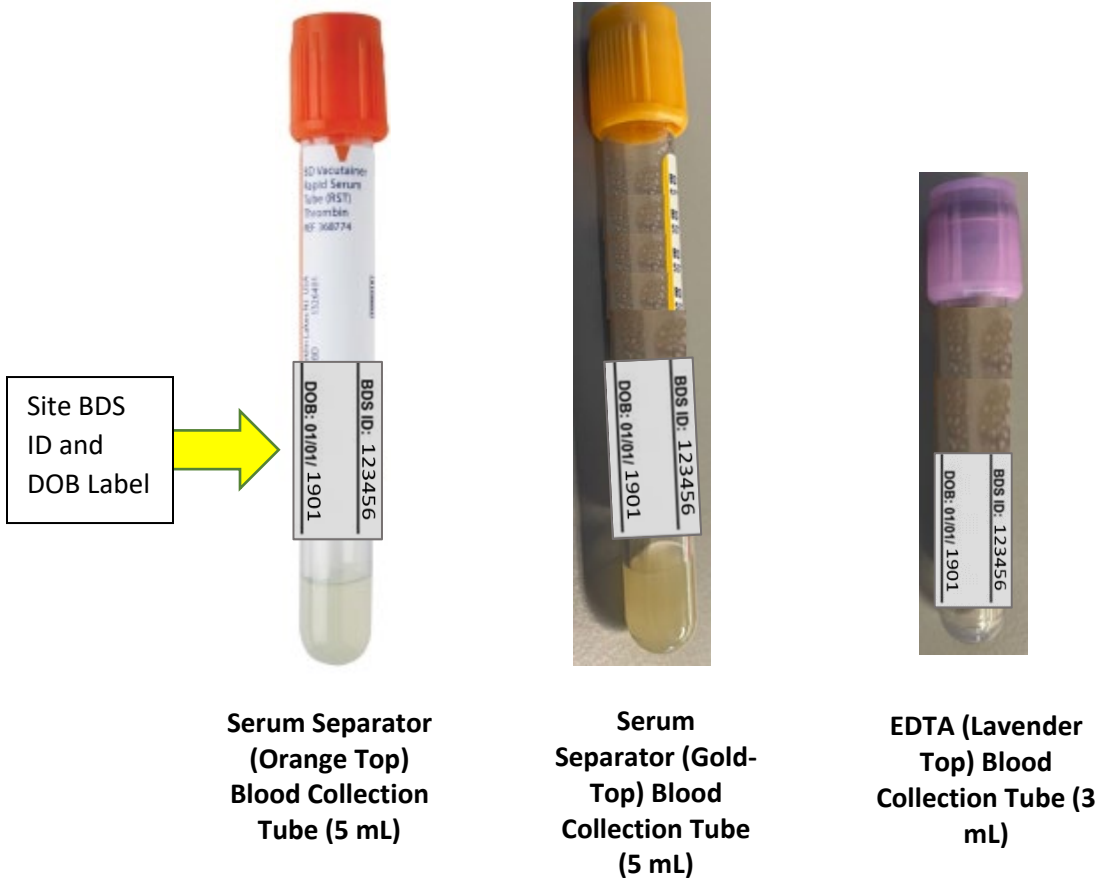
**Each collection tube for NCRAD and UNTHSC will contain two labels:** the Collection and Aliquot Tube Label and the Site and BDS ID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten Site and BDS ID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

**Each NaHep tube for Karyotyping will contain two labels:** the Site and BDS ID label and the kit number label.



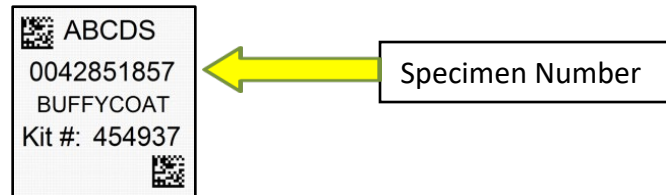
**\*\*Important Note\*\***

**Each collection tube for IU Path Lab will contain one label:** the Site BDS ID and DOB Label. Make sure the subject's DOB is filled in and matches the DOB written on the IU Path Lab Req Form ([Appendix D](#)).

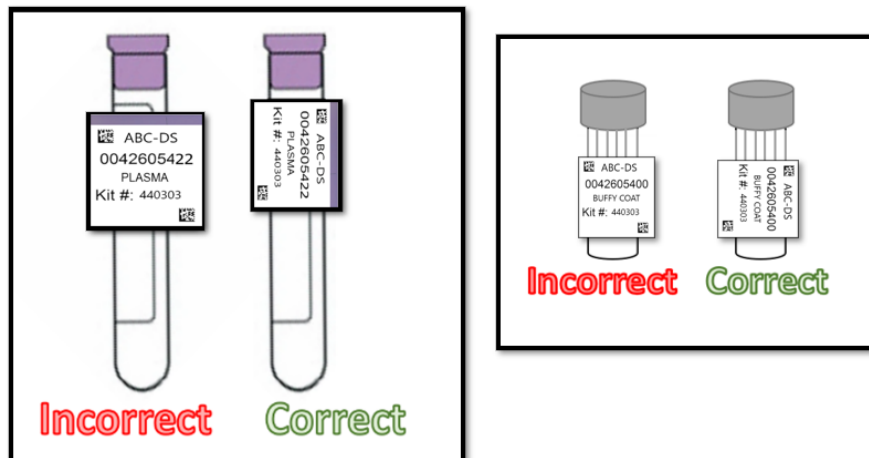


In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place blood collection and aliquot labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing, or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Place cryovials in numerical order based on the specimen number, located near the top of the label. This ensures that no aliquot is misplaced or lost during the shipment process.

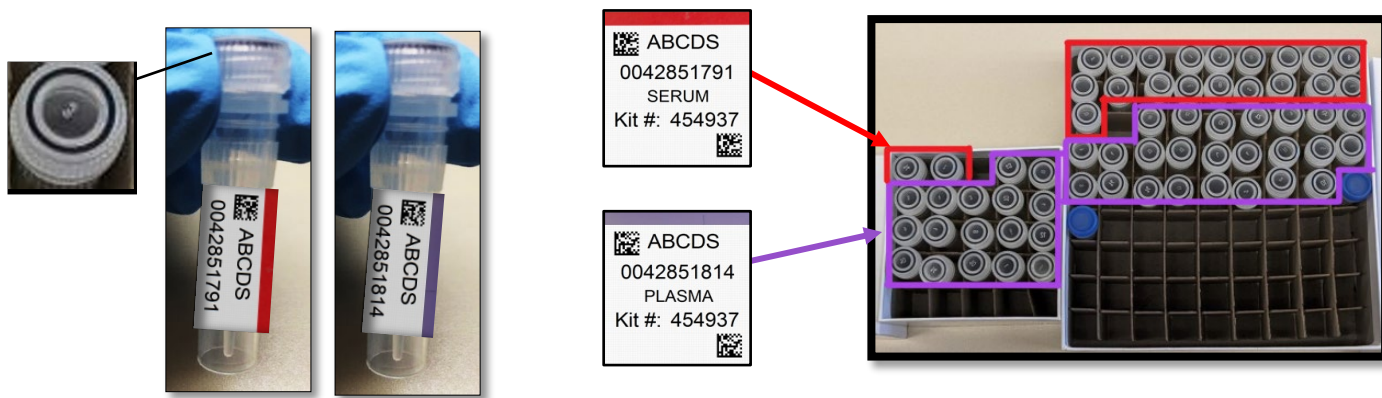


- Using a fine point permanent marker, fill-in and place the Site and BDS ID Labels on the collection tubes only (SST, NaHep, EDTA, RNA) **BEFORE** sample collection, processing, or freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place Site and BDS ID labels on any cryovials.
- The Collection and Aliquot Tube Labels contain a 2D barcode on the top left-hand and bottom right-hand side of the label. Place label **horizontally** on the collection tube (wrapped around sideways if the tube is upright) with either the left or right edge of the label facing towards the tube cap.
- Place Collection and Aliquot Tube Labels on the cryovials in the same manner making sure to place the label **just below the ridges** near the aliquot cap (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.

- **Important Note:** Aliquot Tube Labels for Plasma and Serum are color-coded to replace color coded cap stickers. Cap stickers were causing issues with robotic freezer storage.



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

## 6.2 Video List

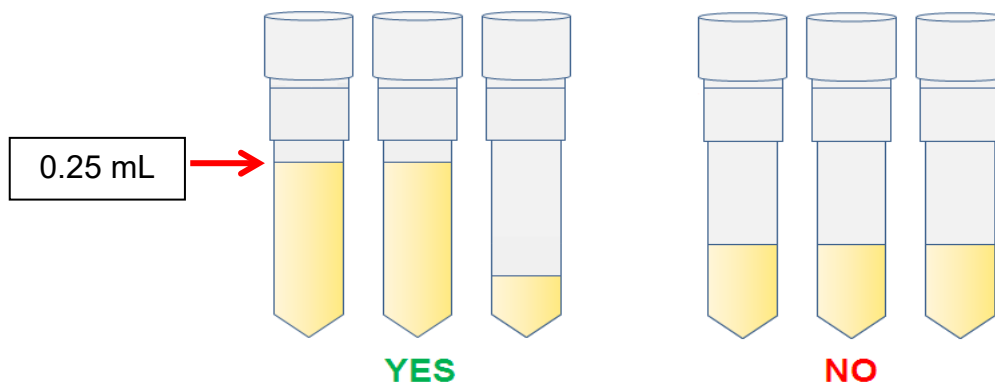
The following training videos are available on the [NCRAD ABC-DS Active Study Page](#) to assist you with the specimen processing, aliquoting, and shipping processes:

- ABC-DS MOP Training
- Frozen Shipping
- Ambient Shipping – *Edit: Place cold pack in refrigerator, not the freezer.*  
See [Section 8.2](#).



### 6.3 Filling Aliquot Tubes (Plasma and Serum)

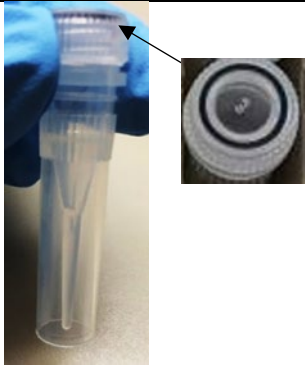

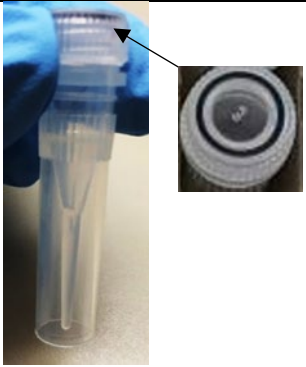





In order to ensure that UNTHSC and NCRAD receive a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each cryovial should be filled to 0.25 milliliters (see picture below) with the respective biological material after processing is completed (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. If there is biologic material remaining that will not fill a subsequent cryovial, that remaining amount should still be included and shipped to NCRAD. You do not have to fill all cryovial tubes provided; you should attempt to fill as many tubes as possible with 0.25 mL of sample. For example, if 3.6 mL of sample is obtained, you should fill 14 cryovial tubes each with 0.25 mL, and one additional cryovial tube with the remaining 0.1 mL.



**Please note:** It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume (anything under 0.25 mL). Please record the specimen number and volume of the residual aliquot on the [Biological Sample and Notification Form \(Appendix B\)](#). If 0.25 mL aliquots unable to be created for UNTHSC, please make note of the low-volume aliquot(s) on the [UNTHSC Intake Form \(Appendix F\)](#).

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

Color Coding	Sample Type
Clear Cap / Red strip on label	Serum
Clear Cap / Purple strip on label	Plasma
Blue Cap	Buffy Coat
Clear Cap	Serum – Clinical Labs from Orange-Top Tube
Red Cap	Serum – Clinical Labs from Gold-Top Tube

 	 			
				
Clear Cap / Red strip on label	Clear Cap / Purple strip on label	Blue Cap	Clear Cap	Red Cap

#### 6.4 Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 2

##### Whole Blood Collection for Isolation of Serum: Serum Separator (Gold-Top) Blood Collection Tube (5 mL) (for processing of serum aliquots).

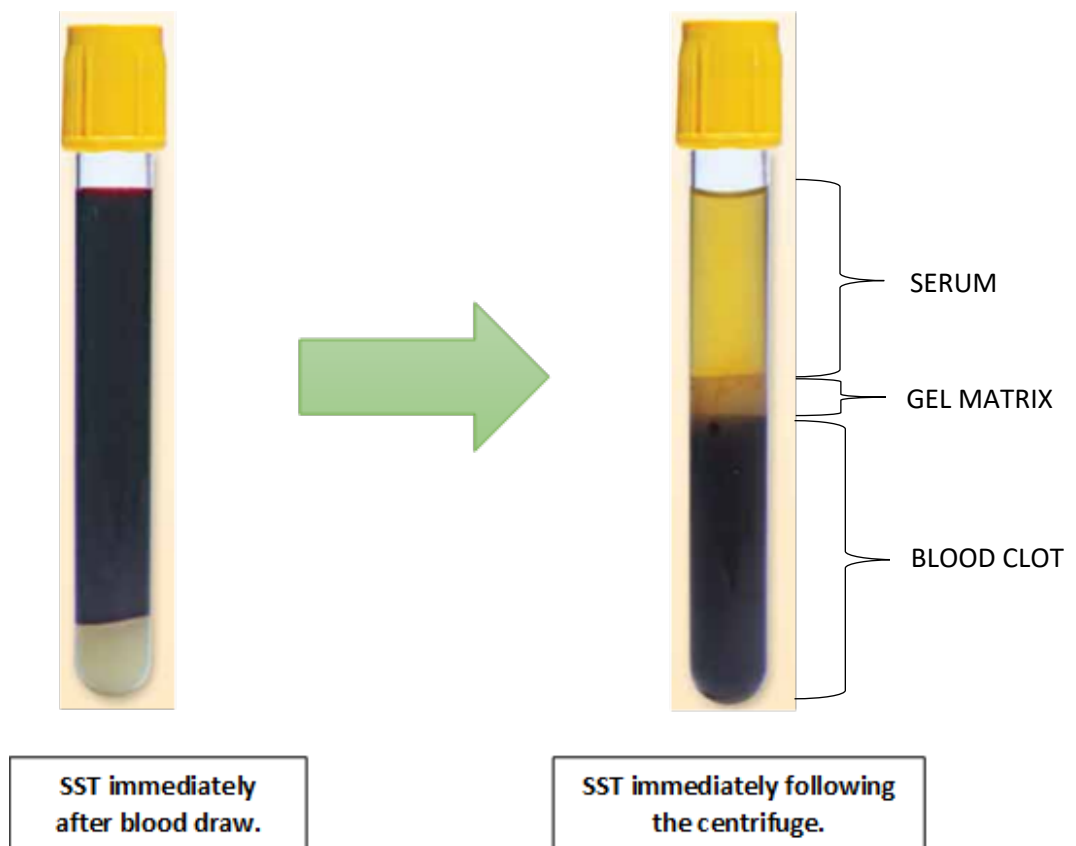
**Important Note:** Ensure all tubes are not expired prior to collection and processing of samples.

1. Set centrifuge to 4°C to pre-chill before use.
2. Place completed Site and BDS ID Label and Collection and Aliquot “**SERUM**” Tube Labels on the Serum Separator (Gold-Top) Blood Collection Tubes (2 x 5 mL). Place pre-printed Aliquot “**SERUM**” Tube Labels with color-coded red strip on the (21) 0.5 mL cryovial tubes with clear caps.
3. Using a blood collection set and a holder, collect blood into **Serum Separator (Gold-Top) Blood Collection Tubes (2 x 5 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 SST at this time. Process blood obtained in existing SST 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. Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.**

- a. **EXCEPTION:** If field-draw, allow blood to clot at room temperature before placing on wet ice, upright on rack and transferring to lab for further processing. Please check “Yes” box on sample form ([Appendix B](#)) if field-draw and make note on [Appendix F](#). If processing takes longer than 2 hours, please make note on both forms.
7. After 30 minutes of clotting, centrifuge the collection tubes for 10 minutes at 2000 RCF (x g) at 4°C. It is critical that the tubes be centrifuged at the appropriate speed to ensure proper serum separation (see worksheet in [Appendix A](#) to calculate RPM.
    - Equivalent rpm for spin at 2000 x g
    - While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form [Appendix B](#).
    - Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection. **EXCEPTION:** If field-draw, place tube on wet ice until you reach the lab for processing.
    - 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. Transfer serum from both gold top Serum Separator tubes into the single 15mL conical tube. Mix the serum by gently inverting the conical tube 3-4 times.
  
9. Using a pipette, transfer plasma from the 15 mL conical tube into the pre-labeled clear-cap "SERUM" cryovials with color-coded red strip. Aliquot 0.25 mL per cryovial (total vials=16-21 with 0.25 mL each). One Gold-Top tube should yield, on average, 5 mL of blood serum. Between both of the Gold-Top tubes, there should be an average of 10 mL of serum, for a total of 16-21 0.5 mL aliquot cryovial tubes per subject with 0.25 mL per cryovial tube. Be sure to only place **serum** in clear-cap cryovials labeled with the "SERUM" label with color-coded red strip. If there is extra serum left, use 1 extra cryovial provided for another <0.25 mL aliquot of serum and label as appropriate. **If a residual aliquot (<0.25 mL) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**



Serum Aliquots (16 to 21 possible)

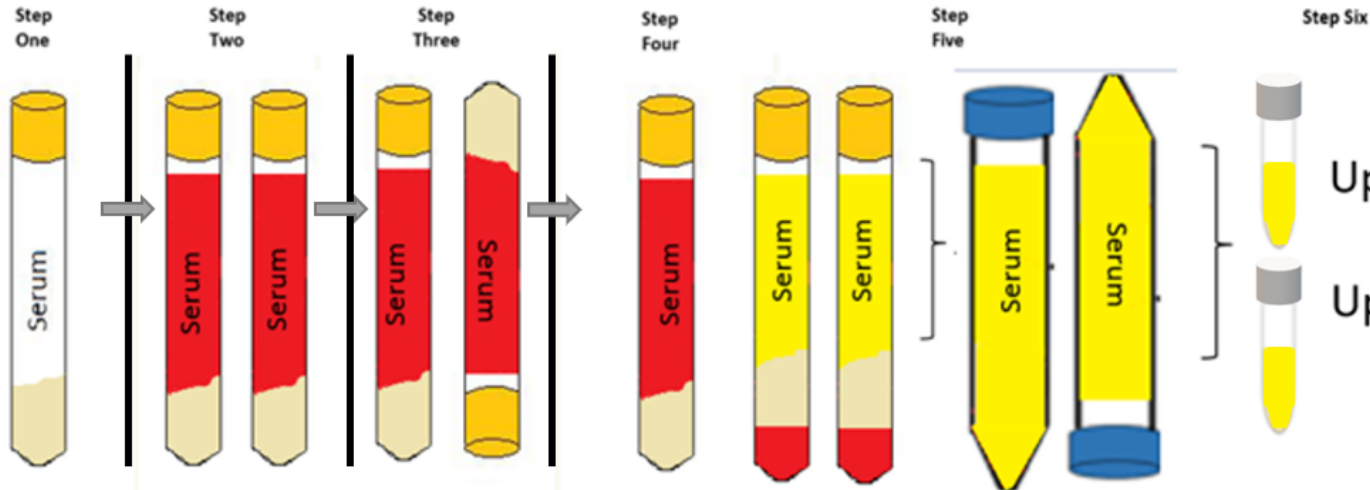


Close up view of 0.5 mL Cryovial



10. Place 2 of labeled cryovials in the 25-cell cryovial box for UNTHSC (**these samples take priority – if only 2 are collected, only send to UNTHSC**) and place the rest (up to 19 cryovials) in the 81-cell cryovial box for NCRAD and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to UNTHSC and NCRAD on dry ice.
11. Dispose of collection tube with gel matrix and red blood cells at the bottom of the tube and empty 15mL conical tube according to your site's guidelines for disposing of biomedical waste.

## Serum Preparation (5 ml Gold-Top Tube) X 2



Up to 19 sent to NCRAD

Up to 2 sent to UNTHSC



- Store tubes at room temperature.
- Place completed Site and BDS ID Label and Collection and Aliquot "SERUM" Tube Labels on 5 mL Gold-Top tubes prior to blood draw.
- Place pre-printed Aliquot "SERUM" Tube Labels with color-coded red strip on the (21) 0.5 mL cryovial tubes with clear caps prior to blood draw.

- Collect blood in (2) 5 mL Gold-Top tubes allowing blood to flow for 10 seconds and ensure blood flow has stopped.

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

- Allow blood to clot for 30 minutes.
- Within 2 hours of blood draw, centrifuge samples at 2000 x g at 4°C for 10 minutes.

- Using a clean transfer pipette, transfer Serum from both 5 mL Gold-Top tubes to the 15 mL conical tube.
- Mix the 15 mL conical tube gently by inverting 3-4 times.

- Aliquot 0.25 mL into each labeled cryovial tube.
- If a residual aliquot is created, document specimen number on Sample Notification Form.
- Store serum aliquots at -80°C until shipment.

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

## 6.5 Sodium Heparin (Green-Top) Blood Collection Tube (4 mL) for karyotyping

### \*\*\*Important Note\*\*\*

If karyotyping has been done for the participant, please check “Yes” on the Biological Sample and Shipment Notification Form ([Appendix B](#)).

NaHep Tube for karyotyping (if not drawn, enter N/A by mL)	
[HHMM] Original volume drawn (1x4 mL NaHep tube):	_____ mL
Has karyotyping ever been completed?	<input type="checkbox"/> Yes <input type="checkbox"/> No
[HHMM] Serum (Serum Separator/Gold Top Tube)	_____ mL

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

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



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

1. **CRITICAL STEP: Store empty Sodium Heparin tube at room temperature, 64°F – 77°F (18°C to 25°C) before use.**
2. Place completed BDS ID Label and Kit Number Label on the green-top NaHep tube.
3. Using a blood collection set and a holder, collect blood into the 4 mL Sodium Heparin tubes using your institution’s recommended procedure for standard venipuncture technique.

#### The following techniques shall be used to prevent possible backflow:

1. Place participant’s arm in a downward position.
  2. Hold tube in a vertical position, below the participant’s arm during blood collection.
  3. Release tourniquet as soon as blood starts to flow into tube.
  4. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
4. Immediately after blood collection, gently invert the tubes 8-10 times to mix sample. **If field-draw**, keep at room temperature until shipping.
  5. Seal the Sodium Heparin tubes in the ambient shipment kit.





6. Fill in the ABC-DS BDS ID and the NaHep tube volume in section 2 of the [Constitutional \(Blood\) Test Requisition Form \(Appendix E\)](#) and fill out the NaHep tube volume on the [Biological Sample Request Form \(Appendix B\)](#) as well. Print out this form and ship with the Sodium Heparin tube.
7. Ship the unprocessed tubes ambient to NCRAD. **Samples must be shipped the same day as collection. Samples must be received the following day after collection. Do NOT draw or ship ambient samples on Friday. Only Monday-Thursday collection and same day shipping.**

## 6.6 EDTA (Lavender-Top) Blood Collection Tube (10 mL) for Plasma and Buffy Coat x 2

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

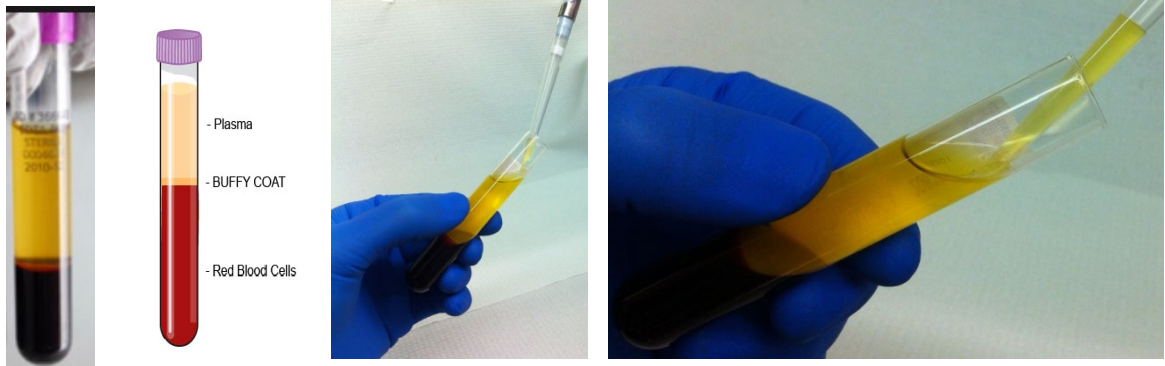
**Important Note:** Ensure all tubes are not expired prior to collection and processing of samples.

1. Set centrifuge to 4°C to pre-chill before use.
2. Place completed Site and BDS ID Label and pre-printed “**PLASMA**” Collection and Aliquot Tube Label on the lavender-top EDTA tube (2 x 10 mL). Place pre-printed Aliquot “**PLASMA**” Tube Labels with color-coded purple strip on the (41) 0.5 mL cryovial tubes with clear caps. Place pre-printed “**BUFFY COAT**” Collection and Aliquot Tube Label on the (2) 2 mL cryovial with a blue lid.
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) x 2** using your institution’s recommended procedure for standard venipuncture technique.

**The following techniques shall be used to prevent possible backflow:**

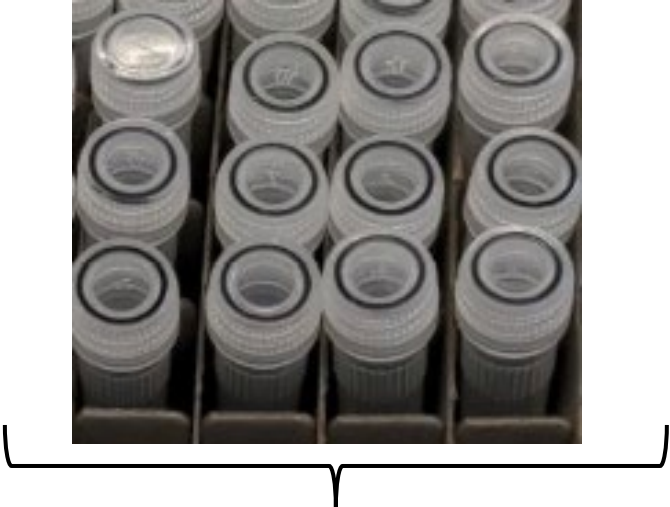
- a. Place donor’s arm in a downward position.
  - b. Hold tube in a vertical position, below the donor’s arm during blood collection.
  - c. Release tourniquet as soon as blood starts to flow into tube.
  - d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
5. 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 tubes 8-10 times.**

7. **CRITICAL STEP: Immediately after inverting the EDTA tubes, place it on wet ice until centrifugation begins.**
  - a. **If field-draw**, keep the samples on wet ice until you reach your destination. Please check “Yes” box on sample form ([Appendix B](#)) if field-draw and make note on [Appendix F](#).
    - 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.** Equivalent rpm for spin at 2000 x g.
    - While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
    - Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection. **If field-draw**, place tube on wet ice until you reach the lab for processing.
    - Record time aliquoted on the Biological Sample Shipment and Notification Form.
8. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall, being careful not to agitate the buffy coat and packed red blood cells at the bottom of the tube (see below). Transfer plasma from both lavender-top EDTA tubes into the single 15mL conical tube. Mix the plasma by gently inverting the conical tube 3 times.
9. Using a pipette, transfer plasma from the 15 mL conical tube into the pre-labeled clear-cap “PLASMA” cryovials with color-coded purple strip. Aliquot 0.25 mL per cryovial (total vials = 35-41 with 0.25 mL each). The EDTA tube should yield, on average, 5 mL of plasma for a total of 35-41 0.5 mL clear-cap cryovial tubes per subject with 0.25 mL per cryovial tube. Be sure to only place **plasma** in clear-cap cryovials labeled with the “PLASMA” label with color-coded purple strip. Take caution not to disturb the red blood cells at the bottom of the tube. If there is extra plasma left, use 1 extra cryovial provided for another <0.25 mL aliquot of plasma. **If a residual aliquot (<0.25 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 15 mL conical tube, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.**

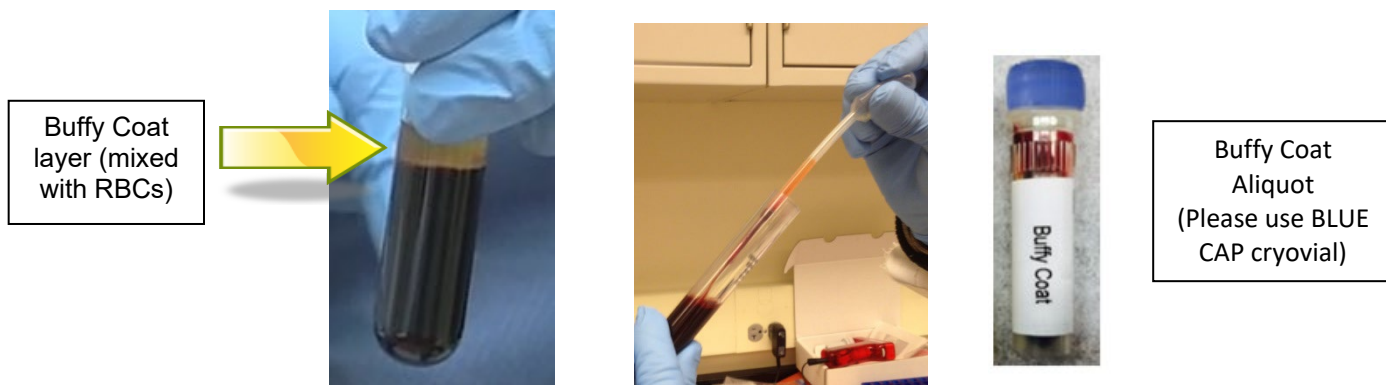
Plasma Aliquots  
(up to 41 possible)



Close up view of 0.5 mL  
Cryovial



10. Place 17 of the labeled cryovials in the 25-cell cryovial box for UNTHSC (**these samples take priority – if only 17 are collected, only send to UNTHSC**) and place the rest (up to 24 cryovials) in the 81-cell cryovial box for NCRAD and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to UNTHSC and NCRAD on dry ice.
  
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 blue cap using a micropipette. The buffy coat aliquots are expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovials with the blue cap and “BUFFY COAT” label.

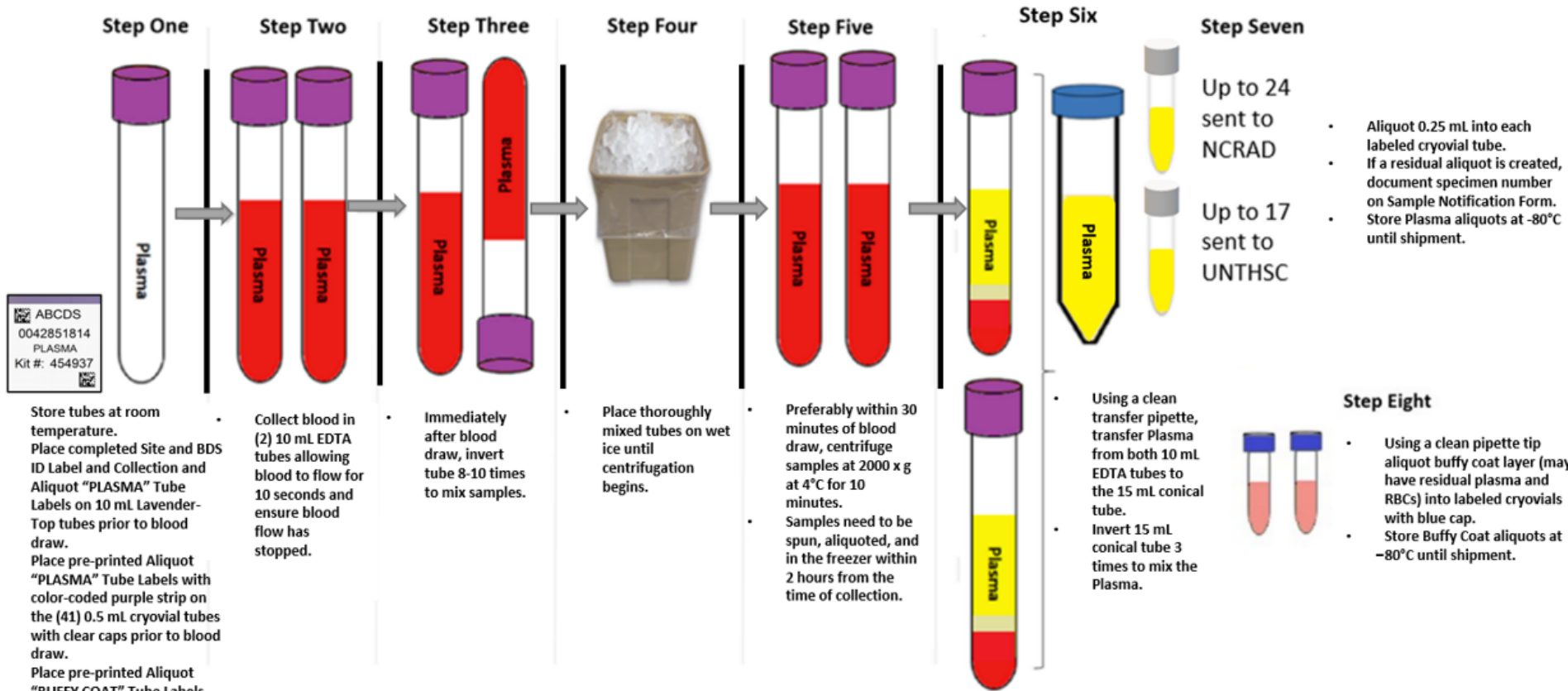


12. Dispose of tubes with red blood cell pellet according to your site’s guidelines for disposing of biomedical waste.
  
13. Place 2 of the labeled buffy coat cryovials in the 81-cell cryovial box for NCRAD and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to UNTHSC and NCRAD on dry ice.

**\*\*\*Important Note\*\*\***

SNP fingerprint is also obtained from every DNA sample, to be compared longitudinally across study visits to identify any subject/sample mix-ups. Apolipoprotein E (APOE) genotype is generated in-house as part of this fingerprint assay.

# Plasma and Buffy Coat Preparation (10 mL Lavender-Top Tube) x 2



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

**6.7 2.5 mL PAXgene™ Tube for RNA x 1****\*\*\*Important Note\*\*\***

In the event the PAXgene™ tube is the only tube being collected at the visit, a 4mL serum discard tube must be drawn first.

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

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

1. Place filled-out Site and BDS ID Label and Collection and Aliquot “RNA” Tube Label on the PAXgene™ tube 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. **CRITICAL STEP: Store PAXgene™ RNA Tubes at room temperature 64°F – 77°F (18°C to 25°C) before use.**
3. Using a blood collection set and a holder, collect blood into the **PAXgene™ RNA Tube** using your institution’s recommended procedure for standard venipuncture technique.

**The following techniques shall be used to prevent possible backflow:**

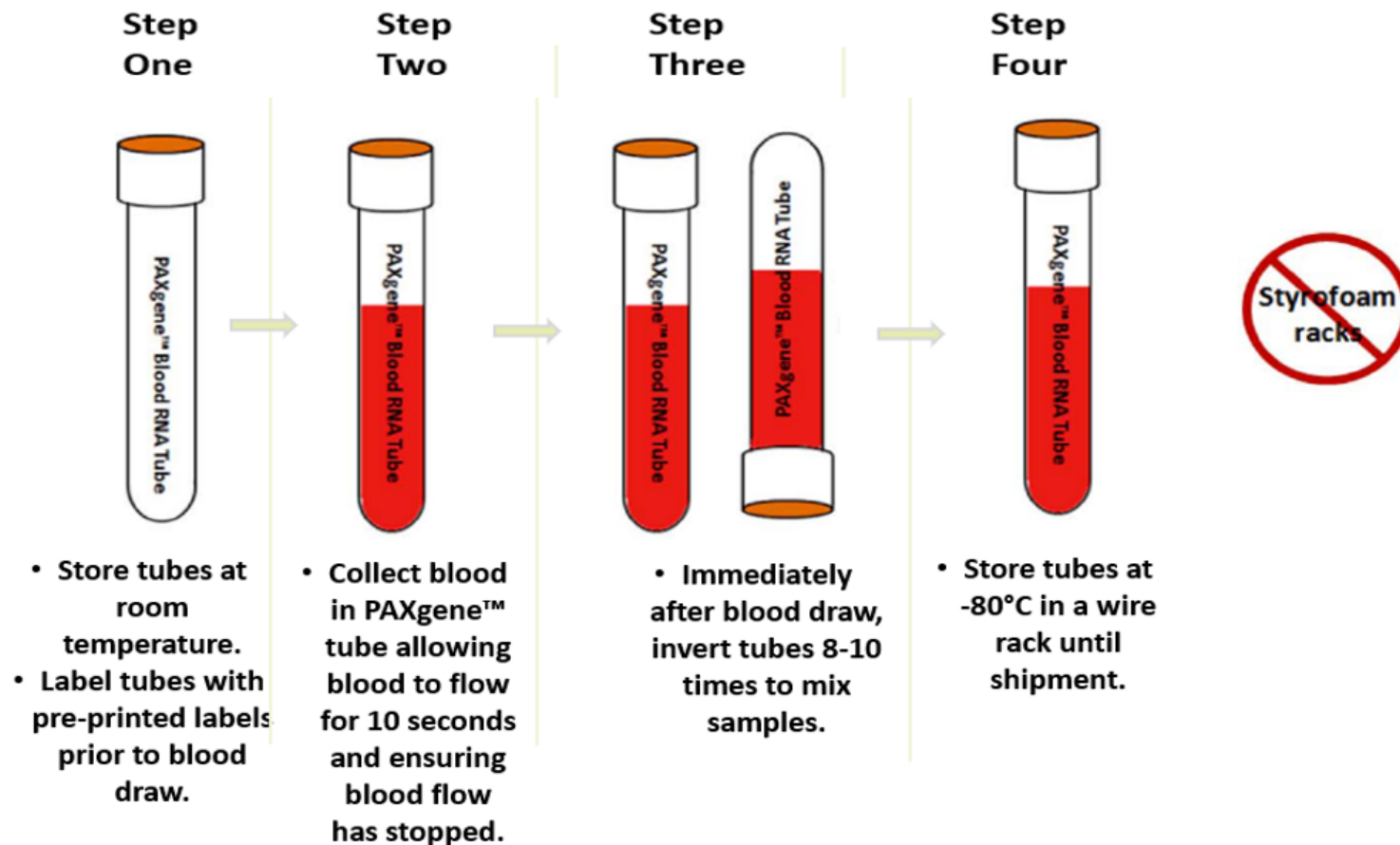
- a. Place participant’s arm in a downward position.
  - b. Hold tube in a vertical position, below the participant’s arm during blood collection.
  - c. Release tourniquet as soon as blood starts to flow into tube.
  - d. Make sure tube additives do not touch the stopper or the end of the needle during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



**80°C freezer.** Complete remainder of the Biological Sample and Shipment Notification Form ([Appendix B](#)). **Please check “Yes” box on sample form (Appendix B) if field-draw and make note on Appendix F.**



# RNA Preparation (2.5mL PAXgene™ Tube) x 1



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

## 6.8 Clinical Labs – IU Path Lab

### 6.8.1 Serum Separator (Orange-Top) Blood Collection Tube (5 mL) for Serum x 1

**Whole Blood Collection for Isolation of Serum: Serum Separator (Orange-Top) Blood Collection Tube (5 mL) for processing of serum aliquots for Free T4, Thyroid, Triiodothyronine, TSH, Vit B12, ATA Preparation.**

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

1. Set centrifuge to 4°C to pre-chill before use.
2. Place completed Site BDS ID and DOB Label on the Serum Separator (Orange-Top) Blood Collection Tubes (1 x 5 mL) and (2) 2.0 mL cryovial tubes with clear caps.
3. Using a blood collection set and a holder, collect blood into **Serum Separator (Orange-Top) Blood Collection Tubes (1 x 5 mL)** using your institution's recommended procedure for standard venipuncture technique.

**The following techniques shall be used to prevent possible backflow:**

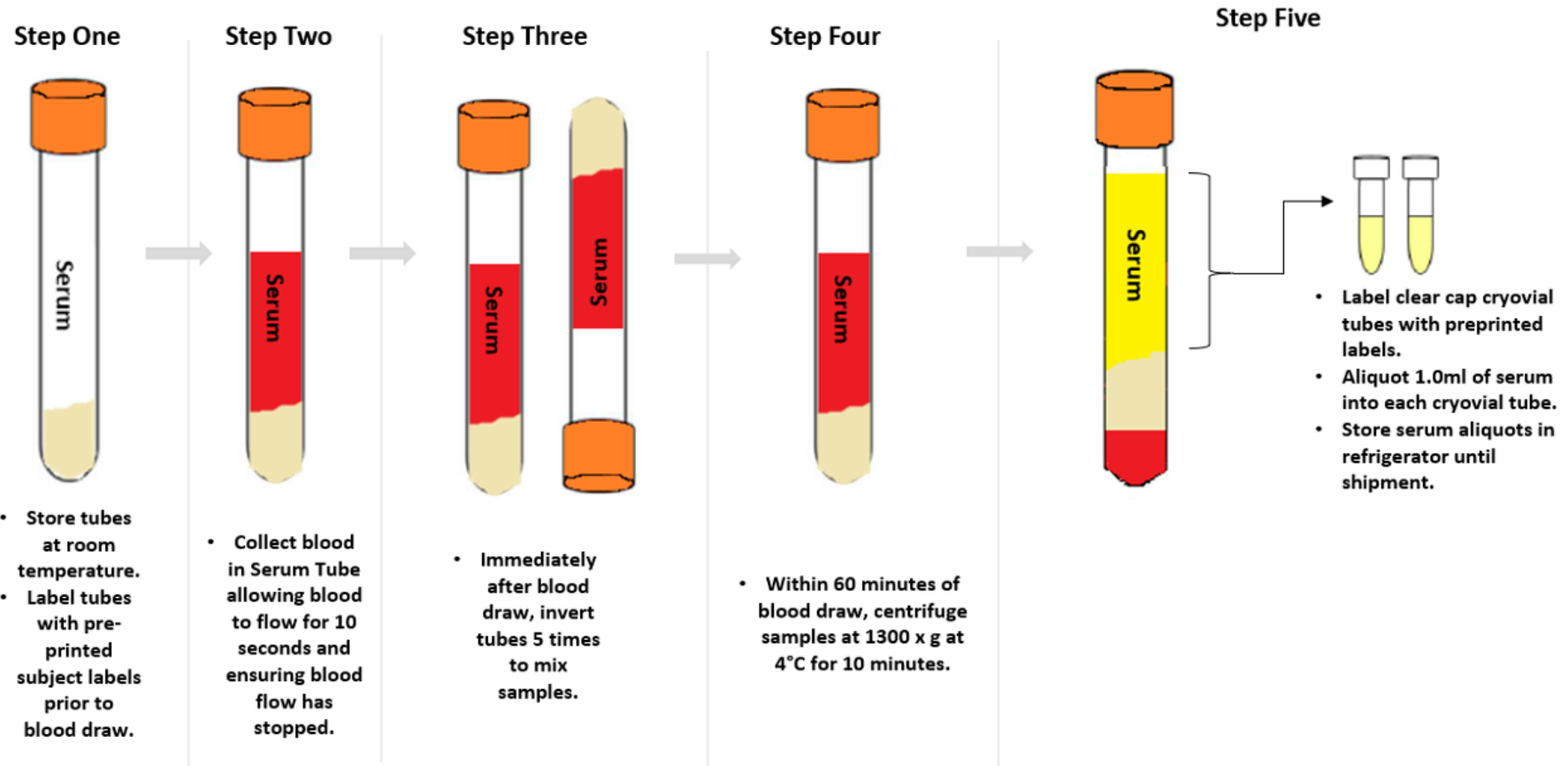
- a. Place donor's arm in a downward position.
  - b. Hold tube in a vertical position, below the donor's arm during blood collection.
  - c. Release tourniquet as soon as blood starts to flow into tube.
  - d. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.
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 [IU Path Lab Req Form \(Appendix D\)](#). Do not attempt to draw an additional SST at this time. Process blood obtained in existing SST tube.
  5. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) tube 5 times.**
  6. **CRITICAL STEP: For best results, serum samples should be spun within 1 hour from the time of collection. EXCEPTION: If field-draw,**



**processing must be completed within 2 hours from time of collection. Place tube on rack in upright position during transfer to lab with cold packs until able to process. Please note on the IU Path Lab form ([Appendix D](#)) that it is a field-draw and the time it takes to process the samples.**

7. Centrifuge the collection tube for 10 minutes at 1300 RCF ( $\times g$ ) 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).**
  - Equivalent rpm for spin at 1300  $\times g$
  - Serum samples need to be spun within 1 hour of collection.

# Free T4, Thyroid, Triiodothyronine, TSH, Vit B12, ATA Preparation (1 X 5 ml Orange Top Tubes)



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

## 6.8.2 Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 1

### Whole Blood Collection for Isolation of Serum: Serum Separator (Gold-Top) Blood Collection Tube (5 mL) (for processing of serum aliquots for Vit D, BMP, Lytes and Lipid Preparation)

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

1. Set centrifuge to 4°C to pre-chill before use.
2. Place completed Site BDS ID and DOB Label on the Serum Separator (Gold-Top) Blood Collection Tubes (1 x 5 mL) and (2) 2.0 mL cryovial tubes with red caps.
3. Using a blood collection set and a holder, collect blood into **Serum Separator (Gold-Top) Blood Collection Tubes (1 x 5 mL)** using your institution's recommended procedure for standard venipuncture technique

#### **The following techniques shall be used to prevent possible backflow:**

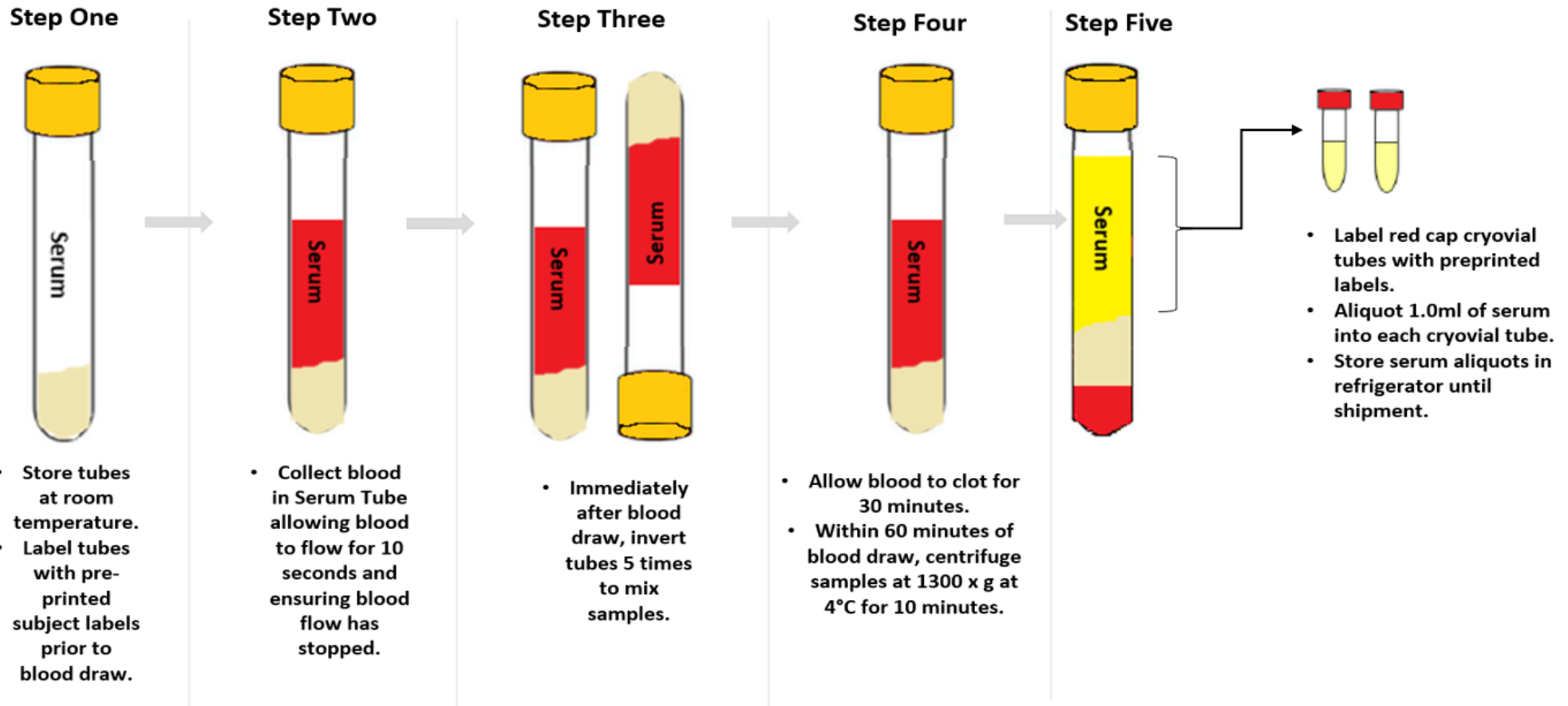
- a. Place donor's arm in a downward position.
  - b. Hold tube in a vertical position, below the donor's arm during blood collection.
  - c. Release tourniquet as soon as blood starts to flow into tube.
  - d. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.
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 [IU Path Lab Req Form \(Appendix D\)](#). Do not attempt to draw an additional SST at this time. Process blood obtained in existing SST tube.
  5. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) 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. For best results, serum samples should be spun within 1 hour from the time of collection. EXCEPTION:** If field-draw, processing must be



**completed within 2 hours from time of collection. Place tube on rack in vertical position during transfer to lab with cold packs until able to process. Please note on the IU Path Lab form ([Appendix D](#)) that it is a field-draw and the time it takes to process the samples.**

7. After 30 minutes of clotting, centrifuge the collection tube for 10 minutes at 1300 RCF (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.**
  - Equivalent rpm for spin at 1300 x g
  - Serum samples need to be spun within 1 hour of collection.

# Vit D, BMP, Lytes and Lipid Preparation (1 X 5 ml Gold Top Tube)



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

### 6.8.3 EDTA (Lavender-Top) Blood Collection Tube (3 mL) x 1

#### Whole Blood Collection for CBC and A1C Preparation: EDTA (Lavender-Top) Blood Collection Tube (3 mL)

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

1. Place filled-out Site BDS ID and DOB Label on the EDTA tube prior to blood draw; no processing is required for this tube. **The single tube is to be shipped to IU Path Lab refrigerated, without processing at the collection site.**
2. **CRITICAL STEP: Store EDTA Tube at room temperature 64°F - 77°F (18°C to 25°C) before use.**
3. Using a blood collection set and a holder, collect blood into the **EDTA (Lavender-Top) Blood Collection Tube (3 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 **EDTA (Lavender-Top) Blood Collection Tube (3 mL)** with its vacuum is designed to draw 3.0 mL of blood into the tube.
  5. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA (Lavender-Top) Blood Collection Tube (3 mL) Tube 8 – 10 times.
  6. Store **EDTA (Lavender-Top) Blood Collection Tube (3 mL)** in refrigerator until shipment. **If field draw, keep tube on cold packs during transfer to lab. Store tube in refrigerator until shipment. Please note on the IU Path Lab form (Appendix D) that it is a field-draw.**



## CBC and A1C Preparation (1 x 3ml EDTA Purple Top Tube)



### Step One



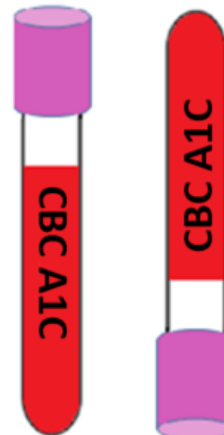
- Store tubes at room temperature.
- Label tubes prior to blood draw.

### Step Two



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

### Step Three



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

### Step Four



- Store whole blood tube in refrigerator until shipment.

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

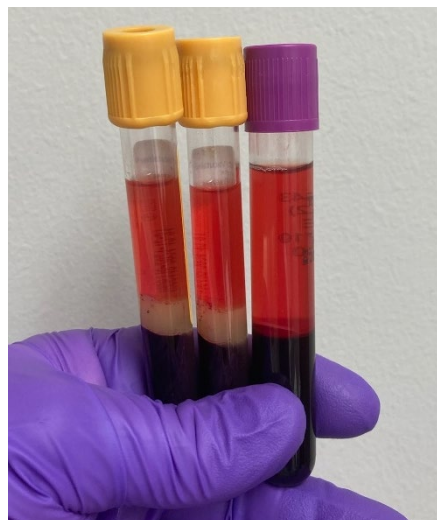
## 7.0 INCOMPLETE OR DIFFICULT BLOOD DRAWS

**\*\*\*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 UNTHSC and NCRAD. See page 11 of the manual for re-draw instructions.**

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

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 UNTHSC and 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
  - d. If samples are hemolyzed (see below), please do not send.



(photo: A.H. – U of Wisconsin)

2. If the biospecimens at a scheduled visit **are not** collected:
  - a. Contact the ABC-DS Monitor and a NCRAD coordinator to alert them of the challenging blood draw or circumstances as to why biospecimens were not collected.
  - b. Schedule participant for a longitudinal visit.
    - i. If samples were unable to be drawn, please draw the Sodium Heparin (Green-Top) Tube for Karyotyping during the next visit (as needed).

## 8.0 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	Tubes to UNTHSC	Ship
Whole blood for isolation of serum	0.25 mL serum aliquot per 0.5 mL cryovial (clear cap)	19	2	Frozen
Whole blood for Karyotyping	N/A	1	0	Ambient
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	0.25 mL plasma aliquot per 0.5 mL cryovial (clear cap)	24	17	Frozen
	1 mL buffy coat aliquot per 2.0 mL cryovial (BLUE CAP)	2	0	Frozen
Whole blood for RNA extraction	N/A	1	0	Frozen

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

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

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

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



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

**8.1 Frozen Shipment Instructions**

1. Contact FedEx (if UW-Madison, contact UPS) to confirm service is available and schedule package to be picked up.
2. On the day of scheduled pick-up, begin packaging specimens on dry ice **at least 1 hour before FedEx/UPS arrives**. Hold samples in -80°C freezer until it is time to package the specimens on dry ice for shipment.

3. Notify NCRAD of shipment by emailing NCRAD coordinators at:  
[alzstudy@iu.edu](mailto:alzstudy@iu.edu)

- Attach the following to the email:
  - Completed Biological Sample and Shipment Notification Form ([Appendix B](#) – also found on the [NCRAD ABC-DS study page](#)).
  - If email is unavailable please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.
  - Please include the tracking number in the body of the email.

Notify UNTHSC of shipment by emailing UNTHSC Lab Manager at:  
[Tori.Como@unthsc.edu](mailto:Tori.Como@unthsc.edu)

- Attach the following to the email:
  - Completed UNTHSC Intake Form ([Appendix F](#) – also found on the [NCRAD ABC-DS study page](#)) and the UNTHSC Import Batch Form ([Appendix G](#)):
    - Aliquot barcodes need to be listed on the UNTHSC Import Batch Form ([Appendix G](#)). NCRAD will send an Excel file with all aliquot barcodes included in each kit when kit supplies are shipped.
  - If email is unavailable please call UNTHSC and do not ship until you've contacted and notified UNTHSC Lab Manager about the shipment in advance.
  - Please include the tracking number in the body of the email.

4. Place all frozen labeled aliquots of serum, plasma and buffy coat in the cryovial cryoboxes.

- i. **FOR NCRAD:** Each cryobox holds 81 samples and there will be approximately 55 cryovial samples being shipped to NCRAD. Please place serum, plasma, and buffy coat aliquots within one cryobox (19 serum, 24 plasma, and 2 buffy coats) per participant blood draw (see below).
- ii. **FOR UNTHSC:** Each cryobox holds 25 samples and there will be approximately 19 cryovial samples being shipped to UNTHSC. Please place plasma and serum aliquots within one cryobox (2 serum, 17 plasma) per participant blood draw (see below).



TO NCRAD: 81-cell cryobox to contain Serum,  
Plasma and Buffy Coat aliquots



TO UNTHSC: 25-cell cryobox to contain  
Serum and Plasma aliquots

- iii. Cryoboxes should contain all of the specimens from the same patient, per time point.
  - iv. **FOR NCRAD - Batch shipping should be performed every 3 months or when specimens from 5 participants accumulates, whichever is sooner.**
  - v. **FOR UNTHSC – Batch shipping should be performed every 3 months or when specimens from 5 participants accumulate, whichever is sooner.**
5. Label the outside of the cryoboxes with the kit number label.
    - i. **FOR NCRAD** - Place serum, plasma and buffy coat aliquots within one cryobox. Place RNA tube in bubble wrap tube sleeve and place within the SAME biohazard bag. These biohazard bags are large enough to contain one cryobox and the RNA tube.
    - ii. **FOR UNTHSC** – Place serum and plasma aliquots within one cryobox in a small biohazard bag.
  6. Place the cryoboxes in the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag.



TO NCRAD: 81-cell cryobox with RNA tube



Place kit number label on each cryobox

TO UNTHSC: 25-cell cryobox to contain Plasma and Serum aliquots

7. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam shipping container.
8. Place the biohazard bag into the provided Styrofoam-lined shipping container on top of the dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container (as pictured below).
9. Fully cover the cryoboxes with approximately 2 inches of dry ice.

Full Shipping Container with Batched Samples and Dry Ice



10. 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.
11. Replace the lid on the Styrofoam carton. Place the completed Biological Sample and Shipment Notification Form for NCRAD or the UNTHSC Intake form





and UNTHSC Import Batch form for UNTHSC in the package on top of the Styrofoam lid for each patient specimen. Close and seal the outer cardboard shipping carton with packing tape.

12. Complete the FedEx return airbill (if UW-Madison, follow UPS instructions provided at site) with the following information:
  - a. Section 1, "From": fill in your name, address, phone number, and Site FedEx Account Number.
  - b. Section 2, "Your Internal Billing Reference": add any additional information required by your site.
  - c. Section 6, "Special Handling and Delivery Signature Options": under "Does this shipment contain dangerous goods?" check the boxes for "Yes, Shipper's Declaration not required" and "Dry Ice". Enter the number of packages (1) x the net weight of dry ice in kg.
  - d. Section 7, "Payment", check sender and bill transportation costs to your site's study FedEx account number.
  
13. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
  - a. Your name and return address
  - b. Net weight of dry ice in kg (must match amount on the airbill)
  - c. Consignee name and address:

<p style="text-align: center;"><b>NCRAD</b> IU School of Medicine 351 West 10<sup>th</sup> Street TK-217 Indianapolis, IN 46202 Phone: 1-800-526-2839</p>
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<p style="text-align: center;"><b>UNTHSC</b> ATTN: Tori Conger 3420 Darcy Street Fort Worth, TX 76107 Phone: 817-735-2638</p>
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- d. Do not cover any part of this label with other stickers, including pre-printed address labels.

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

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



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

**NCRAD**  
IU School of Medicine  
351 West 10<sup>th</sup> Street  
TK-217  
Indianapolis, IN 46202  
Phone: 1-800-526-2839

**UNTHSC**  
ATTN: Tori Conger  
3420 Darcy Street  
Fort Worth, TX 76107  
Phone: 817-735-2638

16. Use FedEx tracking to ensure the delivery occurs as scheduled and is received by NCRAD. Please notify NCRAD by email ([alzstudy@iu.edu](mailto:alzstudy@iu.edu)) and UNTHSC by email at UNTHSC Lab Manager by email ([Tori.Como@unthsc.edu](mailto:Tori.Como@unthsc.edu)) that a shipment has been sent and include the FedEx tracking number in your email.

**\*\*\*Important Note\*\*\***

**FOR NCRAD – For frozen shipments, include no more than five cryovial boxes (separated by patient within 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 INCIPIENT INCLEMENT WEATHER THAT MAY DELAY SHIPMENT/DELIVERY OF SAMPLES**

- Remember to complete the requisition forms and include a copy in your shipment: Biological Sample and Shipment Notification ([Appendix B](#)) for NCRAD and UNTHSC Intake Form ([Appendix F](#)) and UNTHSC Import Batch Form ([Appendix G](#)) for UNTHSC.
- Notify the NCRAD Study Coordinator by email at [alzstudy@iu.edu](mailto:alzstudy@iu.edu) and the UNTHSC contact by email at [Tori.Como@unthsc.edu](mailto:Tori.Como@unthsc.edu) **IN ADVANCE** to confirm the shipment (include FedEx tracking number in email).

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

## 8.2 Ambient Shipping Instructions

### \*\*\*Important Note\*\*\*

**For ambient Sodium Heparin (Green-Top) Blood Collection Tube (1 x 4 mL) shipments, include no more than one tube per shipping container and only include tube from one participant. The ambient NaHep sample must be shipped the day of blood draw.**

**The labeled, unprocessed, sodium heparin NaHep tube will be shipped to NCRAD as outlined below.**

### **IMPORTANT!**

**AMBIENT SAMPLES MUST BE SHIPPED  
MONDAY-THURSDAY ONLY!**

**Do NOT draw blood for ambient shipments on Fridays!**

### \*\*\* Packing and Labeling Guidelines \*\*\*

- The primary receptacle (sodium heparin tube) must be leak proof and must not contain more than 4 mL total.
- The secondary packaging (foam box) must be leak proof.
- Absorbent material must be placed between the primary receptacle (sodium heparin tube) and the secondary packaging (foam box). The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible Person
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373

Ambient Sodium Heparin (Green-Top) Blood Collection Tube (4 mL) shipments should be considered as Category B UN3373 and as such must be triple packaged and compliant with the IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

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

### 8.2.1 NCRAD Packaging and Shipment Instructions (Ambient Shipments)

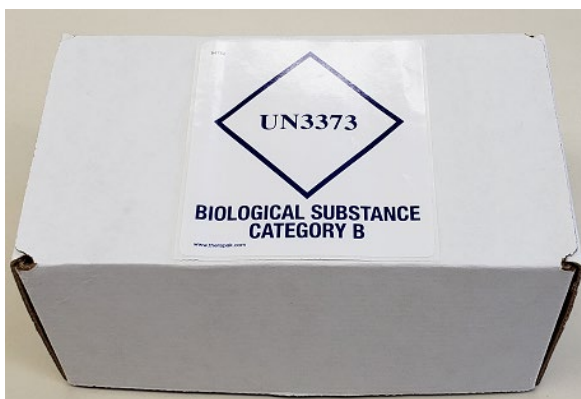
1. Place refrigerant pack in the refrigerator, ~4°C, 24 hours prior to shipment.
2. Contact FedEx (if UW-Madison, contact UPS) to confirm service is available and schedule package to be picked up.
3. Notify NCRAD of shipment by emailing NCRAD coordinators at: [alzstudy@iu.edu](mailto:alzstudy@iu.edu)
  - a. Complete and attach the Constitutional (Blood) Test Requisition Form to the email. (See [Appendix E](#) for an example of the form)
4. Place filled and labeled sodium heparin (green-top) tube within the slots in the absorbent pad provided, and place into the plastic biohazard bag with absorbent sheet. Place the filled out Constitutional (Blood) Test Requisition Form ([Appendix E](#)) inside the biohazard bag as well.



5. Remove as much air as possible from the plastic biohazard bag and ensure the Kit Number Label and BDS ID Label are placed on the tube before sealing the bag according to the directions printed on the bag.
6. Place the sealed biohazard bag inside the cooler and place the refrigerant pack into the cooler on top of the filled biohazard bag.



7. Place the lid onto the cooler.
8. Place the cooler in the provided small IATA Shipping Box.
9. Close shipping box. Label the outside of the cardboard box with the enclosed UN3373 (Biological Substance Category B) label.



10. Place the closed, labeled shipping box within a Clinical Pak. **Seal the Clinical Pak.**
11. Place return airbill on the sealed Clinical Pak.
  - a. Be sure to complete the return airbill with the following information:
    - Section 1, "From": fill in the date, your name, and phone number.
    - Section 2, "Your Internal Billing Reference": add any additional information required by your site.

12. Specimens should be sent to the below address via **FedEx Priority Overnight (or UPS Next Day Air)**. Ambient shipments should be sent Monday through Thursday.

**ABC-DS at NCRAD**

Indiana University School of Medicine  
351 West 10th Street  
TK-217  
Indianapolis, IN 46202

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

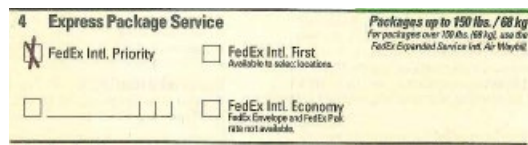
In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by NCRAD for each sample type. Investigators and clinical coordinators for each project are responsible to ensure the requested amounts of each fluid are collected to the best of their ability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

### **8.3 International Shipping**

1. All international shipments will utilize the same packing requirements as specified in [Section 8.1](#) (Frozen Shipping Instructions). **Please note that UNTHSC will not be receiving international shipments. International samples specified for UNTHSC will need to be shipped to NCRAD and will then be forwarded to UNTHSC.**
  - a. International sites will receive a fluorescent label to adhere to the outside of the shipping container so it is clear that NCRAD should forward the international samples to UNTHSC.
2. Two components are necessary for international shipments:
  1. International FedEx return airbill
  2. International Commercial Invoice
3. **By Request if not using World Courier:** NCRAD will provide an International FedEx return airbill to all international sites. However, these international sites are welcome to utilize the FedEx electronic system.
  - a. Be sure to complete the FedEx return airbill with the following information:
    - i. Section 1, From: Enter the date and your name, phone number, complete address, and FedEx account number.
    - ii. Section 2, To: This information will be preprinted with NCRAD's return address and phone number.

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

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



- v. Section 5, Packaging: Please select “Other” for Frozen Shipments and “FedEx Pak” for Ambient Shipments.
- vi. Section 6, Special Handling: Please leave blank.
- vii. Section 7 and 8, Payment: Check Sender and bill transportation costs to your site’s study FedEx account number. Duties and Taxes will also be billed to the sender. If your site requests information to be included as reference, please complete Section 8.
- viii. Section 9, Required Signature: This section must be signed by the sender or department representative.

- b. International Commercial Invoice (See [Appendix C](#) – fillable online PDF [here](#))

- i. The International Commercial Invoice must be completed and placed with the International return airbill.
  1. Include **ONE** original and **THREE** copies of this completed form with the FedEx return airbill.
- ii. Complete “Shipped From” with your name, address, and any additional contact information.
- iii. Complete “Shipped To, Consignee” with the NCRAD shipping address:



**NCRAD**  
 IU School of Medicine  
 351 West 10<sup>th</sup> Street  
 TK-217  
 Indianapolis, IN 46202  
 Phone: 1-800-526-2839

- iv. Complete Number of Packages and Shipping weight to match the information recorded within the International FedEx return airbill.
- v. Immediately below the shipping weight is a section asking for the Country of Origin, Description of Goods, Quantity, Unit Price, and Total Price. Please be as detailed as possible within this section (example pictured below).

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

- vi. Tally the Total Price within the last column for all goods included in shipment and record appropriately.
  - 1. Reminder: the total price/value of the shipment should not exceed \$2,500.
- vii. Complete the final section with your signature.
- viii. Specimens should be sent to the below address via FedEx Priority Overnight. Ambient FedEx shipments should be sent Monday through Thursday. Frozen FedEx Shipments should only be sent Monday through Wednesday.
- ix. Use FedEx tracking to ensure the delivery occurs as scheduled and is received by NCRAD.

#### 8.4 Clinical Labs Shipping

- 1. Notify the IU Pathology Lab of shipment by emailing IU Path Lab study contacts at: [kcleary@IUHealth.org](mailto:kcleary@IUHealth.org), [PJordan@IUHealth.org](mailto:PJordan@IUHealth.org), [rball3@IUHEALTH.ORG](mailto:rball3@IUHEALTH.ORG), AND [jminch1@iuhealth.org](mailto:jminch1@iuhealth.org).
  - a. Attach the following to the email:

- i. Completed IU Path Lab Requisition Form ([Appendix D](#)).
  1. Fill out the following on the form:
    - a. ID in this format: last name = BDS (already printed on template)
    - b. First name = the 7 numerals of the rest of the ABC-DS ID (e.g., 024007).
    - c. Date of collection
    - d. Male/Female
    - e. DOB is required in the system to register the sample. You can use the participant's true DOB or a generic DOB (e.g., 01/01/1950). Either way, the DOB on the req form has to match the DOB on the Site BDS ID and DOB Label.
    - f. MRN: NCRAD will generate this
  - ii. If email is unavailable please call IU Path Lab and do not ship until you've contacted and notified IU Path Lab study contacts about the shipment in advance.
  - iii. Please include the tracking number in the body of the email.
2. Label the 25-slot cryobox with the Site BDS ID and DOB Label.
  - a. **Important Note:** The DOB on the IU Path Lab Req form needs to match the DOB on the Site BDS ID and DOB Label.
3. Place all refrigerated, labeled aliquots of serum from the same subject in the cryobox.
  - a. Each 25-slot cryobox will hold approximately 4 serum samples.

One cryobox containing serum aliquots.



- b. Cryoboxes should contain all specimens from the same participant, per time point.



- c. Place 3mL EDTA tube in bubble wrap tube sleeve and place within the SAME biohazard bag as cryobox containing serum aliquots. These biohazard bags are large enough to contain one cryobox and the EDTA tube.
- d. Place the cryoboxes in the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag.
- e. **Ensure fluorescent round sticker is on biohazard bag.**



- 4. Place biohazard bag within X-Small Insulated shipper with 2 cold packs.
  - a. **CRITICAL STEP: Store Cold Packs in refrigerator, ~4°C, 24-72 hours before use.**
- 5. **Place X-Small Insulated shipper within brown corrugated box and include air pouches.**



- 6. **Place fluorescent rectangular sticker on outside of brown corrugated box.**
- 7. Include original copy of the IU Path Lab Req Form ([Appendix D](#)).
- 8. Seal the outer cardboard shipping carton with packing tape.
- 9. Apply all provided warning labels and the UPS Next Day Air return airbill (**pre-printed and included in the kit**) on the outside of the package. Do not overlap labels.

- a. Ensure the large rectangular fluorescent sticker is on the outside of the brown corrugated box.
- b. Specimens should be sent to the below address via **UPS Next Day Air**. Refrigerated shipments should be sent **Monday through Friday**.

**Important Note:** It is vital to properly notify the IU Path Lab team of sample shipment, especially when shipping on Fridays! The IU Path Lab building is locked on the weekend, therefore one of the staff members will have to let the delivery driver in to complete delivery. Ensure the IU Path Lab requisition form is properly completed, and the tubes properly labeled to avoid verification issues and delayed results.

- c. Schedule a pick-up using the following link: [Schedule a Pickup | UPS - United States](#). You will need to provide the tracking number found on the pre-printed airbill and UPS account number.

**ABC-DS Study at IU Path Lab**  
IU Health Pathology Laboratory  
350 W. 11th Street  
5th Floor, Rm 5013  
Indianapolis, IN 46202

## **9.0 DATA QUERIES AND SAMPLE RECONCILIATION**

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

The Alzheimer's Therapeutic Research Institute (ATRI) data collection team will be collaborating with NCRAD to reconcile information captured in the database compared to samples received and logged at NCRAD. Information that appears incorrect in the ATRI database will be queried through the standard system. Additional discrepancies that may be unrelated to data entry will be resolved with the Principal Investigator in a separate follow up communication. If applicable, a non-conformance report will be provided to sites on a monthly basis.

Results from karyotyping will be uploaded to the ABC-DS EDC site at ATRI by the NCRAD study coordinator 7-10 days after receipt into the laboratory. You can find the results in your site folder: Docs → Site Topics → Choose Site Folder. To set notifications so you know when a report has been uploaded, first go to the "Docs" tab, then click "Manage Notifications" to the right of the search bar. Select a notification for 'file added' or other choices shown.

Clinical lab results will be available through the IU Health Lifepoint application. To access site specific participant results, study personnel must complete an “[Access Request – Lifepoint, IU Non-Employee Form](#)” and submit directly to IU Health. Social Security Number can be documented as “n/a” if the form is signed off on by the Field Site Lead in place of Manager. IU Health will send log-in information to you directly. The ABC-DS Admin Core will not need copies of these set up documents; however, please inform us who from your site will be designated to access the Lifepoint portal.

The ‘group data’ for all participants will be sent from the IU Health Path Lab to LONI, for purposes of analysis. (Site and participant IDs will be removed and new ID assigned per ABC-DS protocol.)

**\*Please check the portal for results ASAP in case a test fails and a re-draw is in order. Saturday deliveries: If issues arise with the specimens, the IU Path Lab will perform the tests offline. The following Monday, after review and corrections, results will be posted.**

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



## **10.0 APPENDICES LIST**

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

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

[Appendix C: International Commercial Invoice](#)

[Appendix D: IU Path Lab Req Form](#)

[Appendix E: Constitutional \(Blood\) Test Requisition Form](#)

[Appendix F: UNTHSC Intake Form](#)

[Appendix G: UNTHSC Import Batch Form](#)



## 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**

**alzstudy@iu.edu**

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



Biospecimen Collection, Processing, and Shipment Manual

### Appendix B

PT ID: \_\_\_\_\_

Site ID: \_\_\_\_\_


Cycle Visit (Circle One):    1    2    3    4

### Sample Collection - Blood & Shipment Notification Form

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

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

To: UNTHSC      Email: Tori.Como@unthsc.edu      Phone: 1-817-735-2638



KIT BARCODE

*General Information:*

From: \_\_\_\_\_ Date: \_\_\_\_\_

Phone: \_\_\_\_\_ Email: \_\_\_\_\_

PT previously enrolled in (circle one):    ADDS    NIAD    N/A-new PT

NIAD/ADDS Legacy ID (if applicable): \_\_\_\_\_ Kit #: \_\_\_\_\_

Arm:     DS Participant     Sibling Control

Sex:     M     F      Year of Birth: \_\_\_\_\_

Shipment Tracking #: \_\_\_\_\_      Field Draw?:     Yes     No

*Blood Collection:*

1. Date Drawn: \_\_\_\_\_ [YYYYMMDD]      2. Time of Draw (24 hour clock): \_\_\_\_\_ [HHMM]

3. Last time subject ate (Date): \_\_\_\_\_ [YYYYMMDD]      4. Last time subject ate (24 hour clock): \_\_\_\_\_ [HHMM]

*Blood Processing:*


RNA PAXgene™ Tube	NaHep Tube for karyotyping (if not drawn, enter N/A by mL)
Original volume drawn (1x2.5mL RNA PAXgene™ tube): _____ mL	Original volume drawn (1x4 mL NaHep tube): _____ mL
Time placed in freezer: _____ [HHMM]	Has karyotyping ever been completed? <input type="checkbox"/> Yes <input type="checkbox"/> No
<b>Plasma (EDTA/Lavender Top Tube)</b>	<b>Serum (Serum Separator/Gold Top Tube)</b>
Time spin started (24 hour clock): _____ [HHMM]	Time spin started (24 hour clock) (30 minutes after draw time): _____ [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 (2x10 mL EDTA tube): EDTA #1: _____ mL    EDTA #2: _____ mL	Original volume drawn (2x5 mL Serum tube): _____ mL
Time aliquoted: _____ [HHMM]	Time aliquoted: _____ [HHMM]
Number of 0.25 mL plasma aliquots created (35-40 total) (Siliconized cryovial): _____ x 0.25 mL	Number of 0.25 mL serum aliquots created (16-20 total) (Siliconized cryovial): _____ x 0.25 mL
<b>Number of 0.25 mL plasma aliquots sent to UNTHSC:</b> _____ <b>Number of 0.25 mL plasma aliquots sent to NCRAD:</b> _____	Number of 0.25 mL serum aliquots sent to UNTHSC: _____      Number of 0.25 mL serum aliquots sent to NCRAD: _____
If applicable, volume of residual plasma aliquot (less than 0.25 mL) (Siliconized cryovial): _____ mL	If applicable, volume of residual serum aliquot (less than 0.25 mL) (Siliconized cryovial): _____ mL
If applicable, specimen number of residual aliquot (last four digits): _____	If applicable, specimen number of residual aliquot (last four digits): _____
Time aliquots placed in freezer (24 hour clock): _____ [HHMM]	Time aliquots placed in freezer (24 hour clock): _____ [HHMM]
Storage temperature of freezer: _____ °C	Storage temperature of freezer: _____ °C
Buffy coat #1 (last four digits): _____      Buffy Coat #1 volume: _____ mL	
Buffy coat #2 (last four digits): _____      Buffy Coat #2 volume: _____ mL	

Notes:

Version 10.2021




### Appendix D: IU Path Lab Req Form

 <b>Indiana University Health</b>		<b>Study/Research Lab Orders</b>		IU Health Pathology Laboratory 350 W. 11th Street, Rm 5013 Indianapolis, IN 46202 317.491.6000 or 800.433.0740 Fax: 317.491.6001	
Patient Name: BDS, _____		DOB 1/1/		Date/Time of Collection	
<input type="checkbox"/> M <input type="checkbox"/> F		MRN Number		PI: Brad Christian	
Client Code:					
<b>Attention IUHPL: Add Cycle to Cerner Comment</b>					
Test Code		Test Name		Select Cycle	
7598	x	1,25 Dihydroxyvitamin D		Cycle 1	Cycle 2
7462	x	Anti-Thyroglobulin Antibody QN		Cycle 1	Cycle 2
6917	x	Basic Metabolic Panel		Cycle 1	Cycle 2
127	x	CBC with Diff		Cycle 1	Cycle 2
6318	x	Hemoglobin A1C HPLC Bld QN		Cycle 1	Cycle 2
6039	x	Lipid Panel SerPI QN		Cycle 1	Cycle 2
6940	x	T4 Free Direct SerPI QN		Cycle 1	Cycle 2
7699	x	Thyroid Peroxidase Ab		Cycle 1	Cycle 2
7430	x	Triiodothyronine Ser QN (T3 Total)		Cycle 1	Cycle 2
7339	x	TSH 3rd Generation SerPI QN		Cycle 1	Cycle 2
6691	x	Vitamin B12 SerPI QN		Cycle 1	Cycle 2




**Appendix E: Constitutional (Blood) Test Requisition Form ([link](#))**

<b>CONSTITUTIONAL (BLOOD) TEST REQUISITION FORM</b>																					
 <p><b>Cytogenetic Laboratories</b>            Indiana University School of Medicine            975 W. Walnut, IB 350, Indianapolis, IN 46202            317/274-2243 (Office) 317/278-1616 (Fax)</p>	<div style="border: 1px solid black; padding: 10px; margin-bottom: 10px;"> <p><i>Patient Laboratory Label</i></p> </div> <p>CAP#: 16789-30    CLLA#: 15D0647198</p>																				
<b>1) PHYSICIAN(S):</b>	<b>FOR LABORATORY USE ONLY:</b>																				
Ordering Physician: <u>Kelley Faber, MS, CCRC</u> Address: <u>MMGE HS 4007</u>  City: <u>Indianapolis</u> State: <u>IN</u> Zip: <u>46202</u> Phone: <u>317-274-7360</u> Fax: _____  Primary Physician: <u>Zoë Potter</u> Address: <u>MMGE HS 4000H</u> City: <u>Indianapolis</u> State: <u>IN</u> Zip: <u>46202</u> Phone: <u>317-278-9086</u> Fax: _____	Date Received: ____/____/____ Time Received: ____:____ am/pm Received By: _____  <div style="text-align: right; font-weight: bold; font-size: 1.2em;">             Account 40-849-19              ABC-DS study           </div> <input type="checkbox"/> BL  <input type="checkbox"/> CMA <input type="checkbox"/> MO <input type="checkbox"/> C-banding <input type="checkbox"/> Q-banding <input type="checkbox"/> NOR-staining Handling Charge x _____ <input type="checkbox"/> Handling <b>ONLY</b> Lab Comment(s): Vacs: ____ green ____ purple; Other _____																				
<b>2) PATIENT INFORMATION:</b>																					
ABC-DS BDS ID: _____ Original volume drawn (1x4 mL NaHep tube): _____ mL																					
<b>4) REFERRING DIAGNOSES ( <i>lease check all that a 1</i> ):</b>																					
<table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> Ambiguous Genitalia</td> <td><input type="checkbox"/> Dysmorphic Features</td> <td><input type="checkbox"/> Seizures</td> <td><input type="checkbox"/> Family History of Chromosome Abnormality</td> </tr> <tr> <td><input type="checkbox"/> Autism Spectrum Disorder</td> <td><input type="checkbox"/> Failure to Thrive</td> <td><input type="checkbox"/> Short Stature</td> <td>(Please provide name, DOB, MRN)</td> </tr> <tr> <td><input type="checkbox"/> Congenital Heart Defect</td> <td><input type="checkbox"/> Hypotonia</td> <td><input checked="" type="checkbox"/> Other <u>ABC-DS Study</u></td> <td></td> </tr> <tr> <td><input type="checkbox"/> Developmental Delay</td> <td><input type="checkbox"/> Multiple Congenital Anomalies</td> <td><input type="checkbox"/> ICD-10 Code: _____</td> <td></td> </tr> <tr> <td><input checked="" type="checkbox"/> Down Syndrome</td> <td><input type="checkbox"/> Recurrent Pregnancy Loss</td> <td></td> <td></td> </tr> </table>		<input type="checkbox"/> Ambiguous Genitalia	<input type="checkbox"/> Dysmorphic Features	<input type="checkbox"/> Seizures	<input type="checkbox"/> Family History of Chromosome Abnormality	<input type="checkbox"/> Autism Spectrum Disorder	<input type="checkbox"/> Failure to Thrive	<input type="checkbox"/> Short Stature	(Please provide name, DOB, MRN)	<input type="checkbox"/> Congenital Heart Defect	<input type="checkbox"/> Hypotonia	<input checked="" type="checkbox"/> Other <u>ABC-DS Study</u>		<input type="checkbox"/> Developmental Delay	<input type="checkbox"/> Multiple Congenital Anomalies	<input type="checkbox"/> ICD-10 Code: _____		<input checked="" type="checkbox"/> Down Syndrome	<input type="checkbox"/> Recurrent Pregnancy Loss		
<input type="checkbox"/> Ambiguous Genitalia	<input type="checkbox"/> Dysmorphic Features	<input type="checkbox"/> Seizures	<input type="checkbox"/> Family History of Chromosome Abnormality																		
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<input checked="" type="checkbox"/> Down Syndrome	<input type="checkbox"/> Recurrent Pregnancy Loss																				
<b>5) REQUESTED TESTING:</b>																					
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> <input checked="" type="checkbox"/> Standard Chromosome Analysis/Karyotype            -- 1 Sodium Heparin Tube (Dark Green-top); 3 mL (infants), 7 mL (adults)   <input type="checkbox"/> Rapid Chromosome Analysis/Karyotype:            -- Preliminary result in 48-72 hours            -- 1 Sodium Heparin Tube (Dark Green-top); 3 mL (infants)   <input type="checkbox"/> Peripheral Blood or Skin Biopsy for Fanconi Anemia Breakage Study using DEB            -- 2 Sodium Heparin Tubes (Dark Green-top); 7-12 mL   <input type="checkbox"/> Standard Chromosome Analysis <u>with</u> Reflex to Microarray (CMA):            -- <u>Reflexes if karyotype is normal.</u>            -- 1 EDTA Tube (Purple-top); minimum 1 mL            -- 1 Sodium Heparin Tube (Dark Green-top); 3 mL (infants), 7 mL (adults)   <input type="checkbox"/> Fluorescence In Situ (FISH) Analysis (<i>Select Probe below</i>)            -- 1 Sodium Heparin Tube (Dark Green-top); 2 mL         </td> <td style="width: 50%; vertical-align: top;"> <input type="checkbox"/> Aneuploidy FISH Full Panel (13, 18, 21, X/Y)  <input type="checkbox"/> Aneuploidy FISH 13/21 Only  <input type="checkbox"/> Aneuploidy FISH 18/X/Y Only            -- Results in 24-72 hours            -- 1 Sodium Heparin Tube (Dark Green-top); 2 mL, minimum 1 mL   <input type="checkbox"/> Constitutional Chromosomal Microarray (CMA) - Peripheral Blood is preferred.  <b>Two tubes of blood are required:</b>            -- 1 EDTA Tube (Purple-top); minimum 1 mL            -- 1 Sodium Heparin Tube (Dark Green-top); minimum 1 mL  <i>Buccal Swabs are also accepted (contact lab for collection kit).</i>   <input type="checkbox"/> Parent/Family Member Studies as Follow-up to CMA            (Test performed based on recommendations in proband's CMA report.)            -- 1 Sodium Heparin Tube (Dark Green-top); 2 mL  <b>Please provide previous patient information (Name, MRN, DOB)</b> </td> </tr> </table>		<input checked="" type="checkbox"/> Standard Chromosome Analysis/Karyotype -- 1 Sodium Heparin Tube (Dark Green-top); 3 mL (infants), 7 mL (adults)  <input type="checkbox"/> Rapid Chromosome Analysis/Karyotype: -- Preliminary result in 48-72 hours -- 1 Sodium Heparin Tube (Dark Green-top); 3 mL (infants)  <input type="checkbox"/> Peripheral Blood or Skin Biopsy for Fanconi Anemia Breakage Study using DEB -- 2 Sodium Heparin Tubes (Dark Green-top); 7-12 mL  <input type="checkbox"/> Standard Chromosome Analysis <u>with</u> Reflex to Microarray (CMA): -- <u>Reflexes if karyotype is normal.</u> -- 1 EDTA Tube (Purple-top); minimum 1 mL -- 1 Sodium Heparin Tube (Dark Green-top); 3 mL (infants), 7 mL (adults)  <input type="checkbox"/> Fluorescence In Situ (FISH) Analysis ( <i>Select Probe below</i> ) -- 1 Sodium Heparin Tube (Dark Green-top); 2 mL	<input type="checkbox"/> Aneuploidy FISH Full Panel (13, 18, 21, X/Y) <input type="checkbox"/> Aneuploidy FISH 13/21 Only <input type="checkbox"/> Aneuploidy FISH 18/X/Y Only -- Results in 24-72 hours -- 1 Sodium Heparin Tube (Dark Green-top); 2 mL, minimum 1 mL  <input type="checkbox"/> Constitutional Chromosomal Microarray (CMA) - Peripheral Blood is preferred. <b>Two tubes of blood are required:</b> -- 1 EDTA Tube (Purple-top); minimum 1 mL -- 1 Sodium Heparin Tube (Dark Green-top); minimum 1 mL <i>Buccal Swabs are also accepted (contact lab for collection kit).</i>  <input type="checkbox"/> Parent/Family Member Studies as Follow-up to CMA (Test performed based on recommendations in proband's CMA report.) -- 1 Sodium Heparin Tube (Dark Green-top); 2 mL <b>Please provide previous patient information (Name, MRN, DOB)</b>																		
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<b>6) MICRODELETION FISH ANALYSIS REQUESTED:</b>																					
<table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> Angelman</td> <td><input type="checkbox"/> Kallman</td> <td><input type="checkbox"/> Smith-Magenis</td> <td><input type="checkbox"/> Williams</td> </tr> <tr> <td><input type="checkbox"/> Cri-Du Chat</td> <td><input type="checkbox"/> Miller-Dieker</td> <td><input type="checkbox"/> SRY</td> <td><input type="checkbox"/> Wolf-Hirschhorn</td> </tr> <tr> <td><input type="checkbox"/> DiGeorge (VCFS)</td> <td><input type="checkbox"/> Prader-Willi</td> <td><input type="checkbox"/> STS</td> <td></td> </tr> </table>		<input type="checkbox"/> Angelman	<input type="checkbox"/> Kallman	<input type="checkbox"/> Smith-Magenis	<input type="checkbox"/> Williams	<input type="checkbox"/> Cri-Du Chat	<input type="checkbox"/> Miller-Dieker	<input type="checkbox"/> SRY	<input type="checkbox"/> Wolf-Hirschhorn	<input type="checkbox"/> DiGeorge (VCFS)	<input type="checkbox"/> Prader-Willi	<input type="checkbox"/> STS									
<input type="checkbox"/> Angelman	<input type="checkbox"/> Kallman	<input type="checkbox"/> Smith-Magenis	<input type="checkbox"/> Williams																		
<input type="checkbox"/> Cri-Du Chat	<input type="checkbox"/> Miller-Dieker	<input type="checkbox"/> SRY	<input type="checkbox"/> Wolf-Hirschhorn																		
<input type="checkbox"/> DiGeorge (VCFS)	<input type="checkbox"/> Prader-Willi	<input type="checkbox"/> STS																			

## Appendix F: UNTHSC Intake Form ([link](#))

**\*Click link to view all pages\***


Version: 2021-10-13

### UNTHSC Sample Shipping Process

We appreciate your time and dedication to this project; with that, we want to ensure the best scenario for your samples upon arrival and best possible test results.

Our testing is a highly automated process requiring a good deal of preparation prior to any testing. In order for the Institute for Translational Research Laboratory to be prepared for the upcoming shipment of your samples, we ask that you answer a few questions regarding your samples as this will prevent any delay in obtaining your results.

**\*\*\*MINIMUM VOLUME REQUIREMENT\*\*\* 500ul of sample for MSD and 500ul of sample for Quanterix. Please discuss this with our lab personnel.**

Please be sure to include:

- An excel file with the 5 columns listed below-
  - Unique Sample ID (Each sample is uniquely identified)- **required**
  - Unique TubeID/Barcode-**required**
  - Visit # (unique timepoint for each sample in the study)-**required for multiple visits**
  - Date of Collection- if applicable
  - Notes for sample (i.e. hemolyzed etc)- if applicable

Unique Sample ID	Unique Tube ID/Barcode	Visit Number	Date of Collection	Notes for Samples

- Indicate sample type(s) to be sent
  - Plasma
  - Serum
  - Other \_\_\_\_\_
- Number of samples per sample type \_\_\_\_\_
- Volume of each sample (please add notes for any low volume samples).
  - Please note, any sample we declare as **unusable will be discarded**.

Page 1 of 4



**ADDENDUM 1 MOM’S SUBSTUDY – PLASMA, DNA, AND SERUM**

This section provides collection, processing and shipment information for sites that are participating in the MOM’s Substudy. The Plasma, Buffy Coat for DNA extraction, and Serum collection for a subset of participants’ parent will be conducted on a different calendar day than the ABC-DS parent protocol study visit.

**11.0 SERUM, PLASMA, AND DNA COLLECTION FOR SUBSET OF PARTICIPANTS**

**11.1 Site Required Equipment**

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

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquet
- Alcohol Prep Pad
- Gauze Pad
- Bandage
- Butterfly needles (21 gauge) and hub
- Microcentrifuge tube rack
- Sharps bin and lid
- Wet Ice Bucket

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:

- For UNTHSC: 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)

**11.2 Biospecimen Collection Schedule**

ABC-DS Blood-Based Biomarker Collection Schedule for Parents:

	Serum	Plasma	DNA
<b>One Visit:</b>	X	X	X
<b>SHIP TO:</b>	UNTHSC	UNTHSC	UNTHSC

\*Collection will be at 1 time-point for all parents.

Whole blood is collected in two types of tubes (5mL gold-top serum separator tube, 10mL lavender-top EDTA tube).

- The (1) 5mL gold top serum separator tubes are processed locally into serum fractions. They are then aliquoted, frozen at the study site and shipped to UNTHSC.
- The (1) 10mL EDTA is processed locally into plasma and buffy coat fractions. They are then aliquoted, frozen at the study site and shipped to UNTHSC.

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 14.1](#). Guidelines for the processing, storage location, and timing of sample collection are listed in the tables below.

### 11.3 Biospecimen Collection Chart

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to UNTHSC	Ship
Whole blood for isolation of serum	<b>Serum Separator (Gold-Top) Blood Collection Tube (5 mL)</b>	1	N/A	N/A	N/A
	SERUM: 0.5 mL cryovials	7	0.25 mL serum aliquot per 0.5 mL cryovial (clear cap)	7	Frozen
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	<b>EDTA (Lavender-Top) Blood Collection Tube (10 mL)</b>	1	N/A	N/A	N/A
	PLASMA: 0.5 mL cryovials	17	0.25 mL plasma aliquot per 0.5 mL cryovial (clear cap)	17	Frozen
	BUFFY COAT: 2.0 mL cryovials	1	1 mL buffy coat aliquot per 2.0 mL cryovial (BLUE CAP)	1	Frozen

## 12.0 SPECIMEN COLLECTION KITS, SHIPPING KITS, AND SUPPLIES

NCRAD will provide: 1) blood collection kits for specimens to be stored at UNTHSC; 2) frozen shipping kits for shipping specimens to UNTHSC (with the exception of dry ice and equipment supplies listed in Section 11.1) and 3) supplemental supplies. These materials include blood tubes, pipettes, pipette tips, boxes for serum/plasma/buffy coat aliquots, as well as partially completed shipping labels to send materials to UNTHSC. Kit Number Labels, Site and BDS ID Labels, Collection and Aliquot Tube Labels will all be provided by NCRAD. Collection and Aliquot Tube Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to [Section 13.1](#).

### 12.1 Specimen Collection Kit Contents

Collection kits contain the following (for each 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.*

#### Substudy Blood Collection Kit

Quantity	ABC-DS MOM's Substudy Blood Kit
1	Serum Separator (Gold-Top) Blood Collection Tube (5 mL)
1	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
7	Cryovial tube (0.5 mL) with clear cap (Serum)
17	Cryovial tube (0.5 mL) with clear cap (Plasma)
1	Cryovial tube (2.0 mL) with blue cap
2	Disposable graduated transfer pipette (1 mL)
27	Pre-printed Collection and Aliquot Tube Label
3	Pre-printed Kit Number Label
3	Labels for handwritten Site and BDS ID
1	Resealable bag
1	Microcentrifuge tube box (holds up to 25 microcryovials)

#### Substudy Blood Supplemental Kit

Quantity	ABC-DS MOM's Substudy Blood Supplemental Kit
4	Serum Separator (Gold-Top) Blood Collection Tube (5 mL)
4	EDTA (Lavender-Top) Blood Collection Tube (10 mL)
54	Cryovial tube (0.5 mL) with clear cap
4	Cryovial tube (2.0 mL) with blue cap
6	Disposable graduated transfer pipette (1 mL)
5	Labels for handwritten Site and BDS ID
1	Resealable bag
1	Microcentrifuge tube box (holds up to 25 microcryovials)

**Frozen Blood Shipping Kit**

Quantity	MOM’s Substudy Frozen Blood Shipping Kit
5	Plastic Biohazard bag with absorbent sheet (small)
1	Shipping airbill
1	Resealable bag
1	Shipping box/Styrofoam container
1	Warning label packet with dry ice sticker

**12.2 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 [ABC-DS Kit Request System](#) to request additional kits and follow the prompts to request the desired supplies. Options include ordering a specific number of kits; we are also including the option of simply ordering the desired amount of extra supplies.

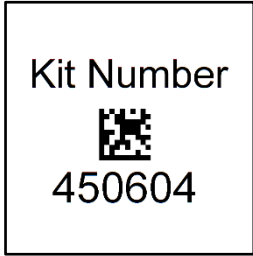
Please allow **TWO - THREE weeks** for kit orders to be processed and delivered.

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

**13.0 BLOOD COLLECTION AND PROCESSING PROCEDURES**

**13.1 Labeling Samples**

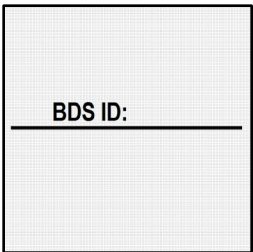
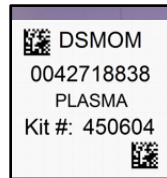
<p align="center"><b>**Label Type Summary**</b></p> <ol style="list-style-type: none"> <li>1. Kit Number Label</li> <li>2. Collection and Aliquot Tube Label</li> <li>3. Site and BDS ID Label</li> </ol>
-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------



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 for UNTHSC.



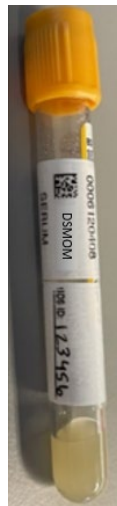
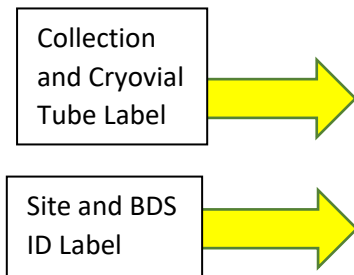
The **Collection and Aliquot Tube Labels** for blood derivatives are placed on all collection and aliquot tubes for UNTHSC. **Note:** *Aliquot Tube Labels for Plasma and Serum are color-coded to replace cap stickers.*



The **Site and BDS ID Labels** are placed on all blood collection tubes for UNTHSC and NCRAD.

**\*\*Important Note\*\***

**Each collection tube will contain two labels:** the Collection and Aliquot Tube Label and the Site and BDS 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 BDS ID label below.



**Serum Separator  
(Gold-Top) Blood  
Collection Tube (5 mL)**

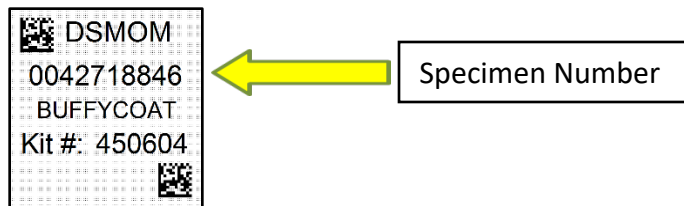


**EDTA (Lavender-Top)  
Blood Collection Tube  
(10 mL)**

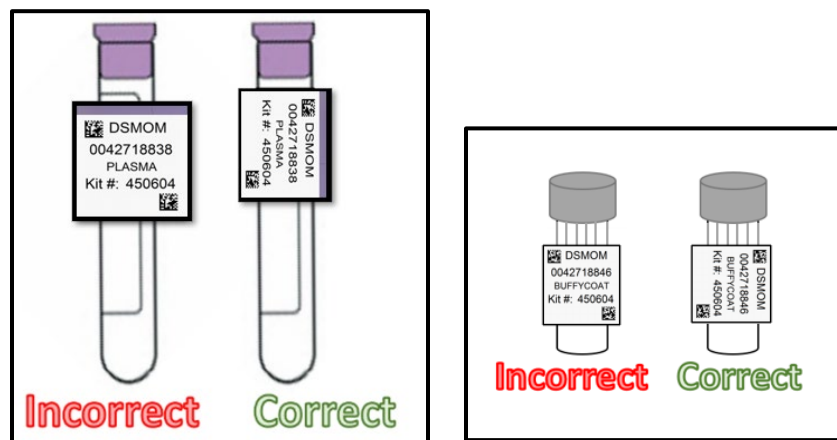


In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place blood collection and aliquot labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing, or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Place cryovials in numerical order based on the specimen number, located near the top of the label. This ensures that no aliquot is misplaced or lost during the shipment process

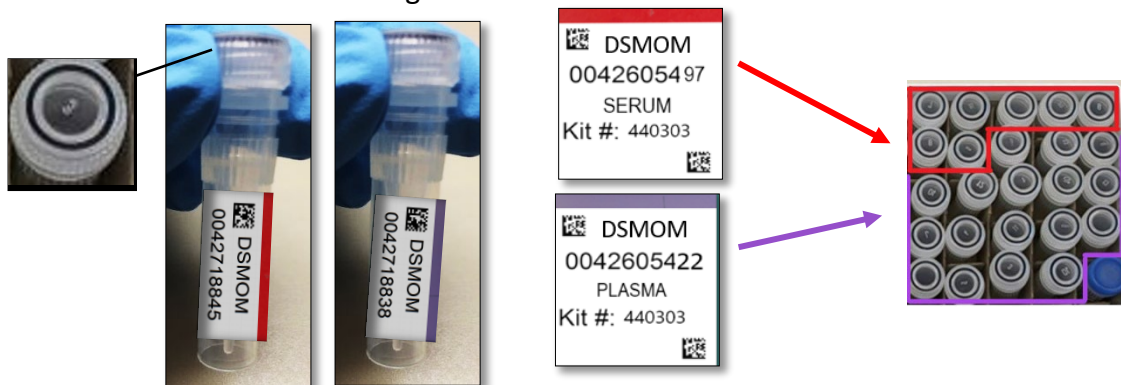


- Using a fine point permanent marker, fill-in and place the Site and BDS ID Labels on the collection tubes only (SST, EDTA) **BEFORE** sample collection, processing, or freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place Site and BDS ID labels on any cryovials.
- The Collection and Aliquot Tube Labels contain a 2D barcode on the top left-hand and bottom right-hand side of the label. Place label **horizontally** on the collection tube (wrapped around sideways if the tube is upright) with either the left or right edge of the label facing towards the tube cap.
- Place Collection and Aliquot Tube Labels on the cryovials in the same manner making sure to place the label **just below the ridges** near the aliquot cap (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.

- **Important Note:** Aliquot Tube Labels for Plasma and Serum are color-coded to replace color coded cap stickers. Cap stickers were causing issues with robotic freezer storage.



- 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.

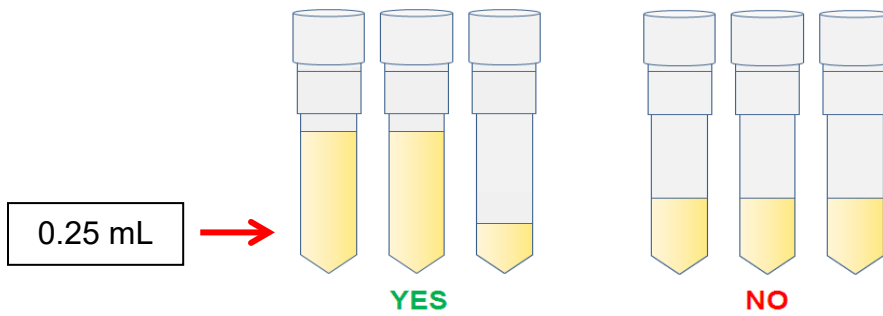
### 13.2 Video List

The following training videos are available on the [NCRAD ABC-DS Active Study Page](#) to assist you with the specimen processing, aliquoting, and shipping processes:

- MOM's Study MOP Training
  - Frozen Shipping

### 13.3 Filling Aliquots

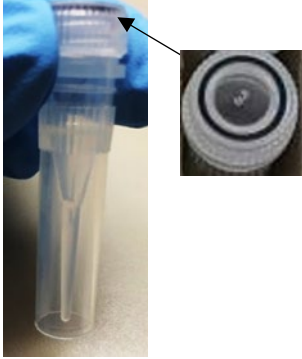


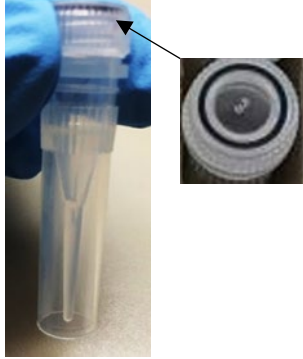



In order to ensure that UNTHSC receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each cryovial should be filled to 0.25 milliliters (see picture below) with the respective biological material after processing is completed (refer to 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. If there is biologic material remaining that will not fill a subsequent cryovial, that remaining amount should still be included and shipped to UNTHSC. You do not have to fill all cryovial tubes provided; you should attempt to fill as many tubes as possible with 0.25 mL of sample. For example, if 3.6 mL of sample is obtained, you should fill 14 cryovial tubes each with 0.25 mL, and one additional cryovial tube with the remaining 0.1 mL.



**Please note:** It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume (anything under 0.25 mL). Please record the specimen number and volume of the residual aliquot on the [UNTHSC Intake Form \(Appendix F\)](#).

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

Color Coding	Sample Type
Clear Cap / Red strip on label	Serum
Clear Cap / Purple strip on label	Plasma
Blue Cap	Buffy Coat

  	  	
<b>Clear Cap / Red strip on label</b>	<b>Clear Cap / Purple strip on label</b>	<b>Blue Cap</b>

### 13.4 Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 1

#### Whole Blood Collection for Isolation of Serum: Serum Separator (Gold-Top) Blood Collection Tube (5 mL) (for processing of serum aliquots).

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

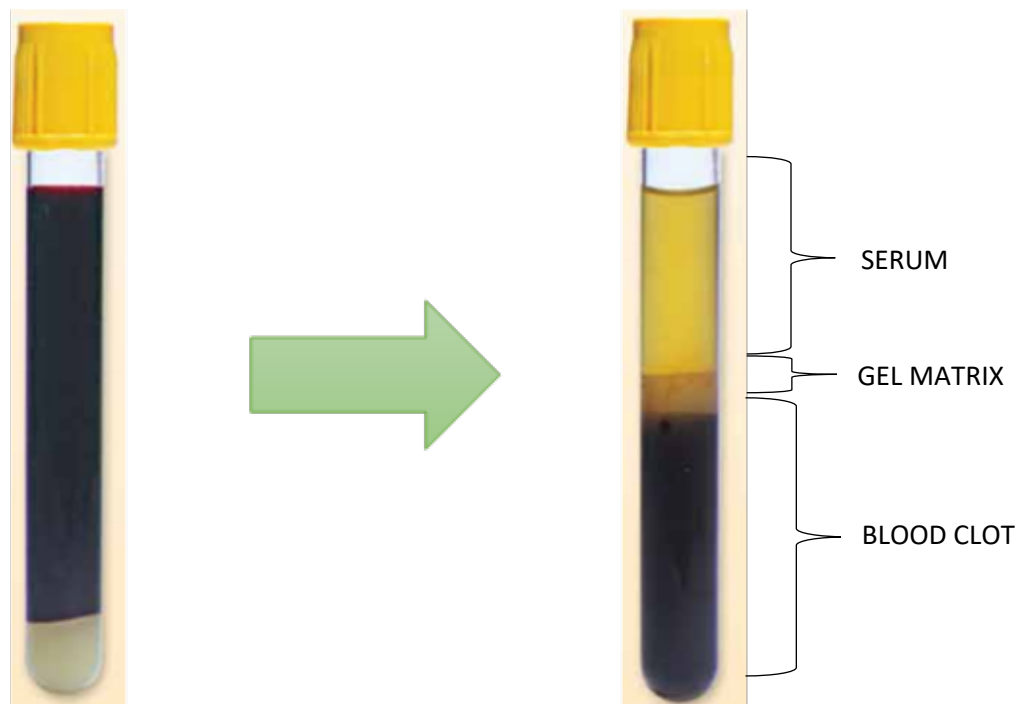
1. Set centrifuge to 4°C to pre-chill before use.
2. Place completed Site and BDS ID Label and Collection and Aliquot **“SERUM”** Tube Labels on the Serum Separator (Gold-Top) Blood Collection Tubes (1 x 5 mL). Place pre-printed Aliquot **“SERUM”** Tube Labels with color-coded red strip on the (7) 0.5 mL Cryovial tubes with clear caps.
3. Using a blood collection set and a holder, collect blood into **Serum Separator (Gold-Top) Blood Collection Tube (1 x 5 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 ‘UNTHSC Intake Form’. Do not attempt to draw an additional SST at this time. Process blood obtained in existing SST 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. Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.**
    - a. **EXCEPTION:** If field-draw, allow blood to clot at room temperature before placing on wet ice, upright on rack and transferring to lab for

**further processing. Please make note on [Appendix F](#) if field draw. If processing takes longer than 2 hours, please make note on form.**

7. After 30 minutes of clotting, centrifuge the collection tube for 10 minutes at 2000 RCF (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).**
  - Equivalent rpm for spin at 2000 x g
  - Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection. **EXCEPTION:** If field-draw, place tube on wet ice until you reach the lab for processing.



**SST immediately  
after blood draw.**

**SST immediately following  
the centrifuge.**

8. Remove the serum from the 5mL SST tube by tilting the tube and placing the pipette tip along the lower side of the wall. Transfer plasma into the pre-labeled clear-cap “SERUM” cryovials with color-coded red strip. Aliquot 0.25 mL per cryovial (total vials=7 with 0.25 mL each). One Gold-Top tube should yield, on average, 5 mL of blood serum. Be sure to only place **serum** in Cryovials labeled with the “SERUM” label with color-coded red strip. If there is extra serum left, use 1 extra cryovial provided for another <0.25 mL aliquot of serum and label as appropriate. **If a residual aliquot (<0.25 mL) is created,**

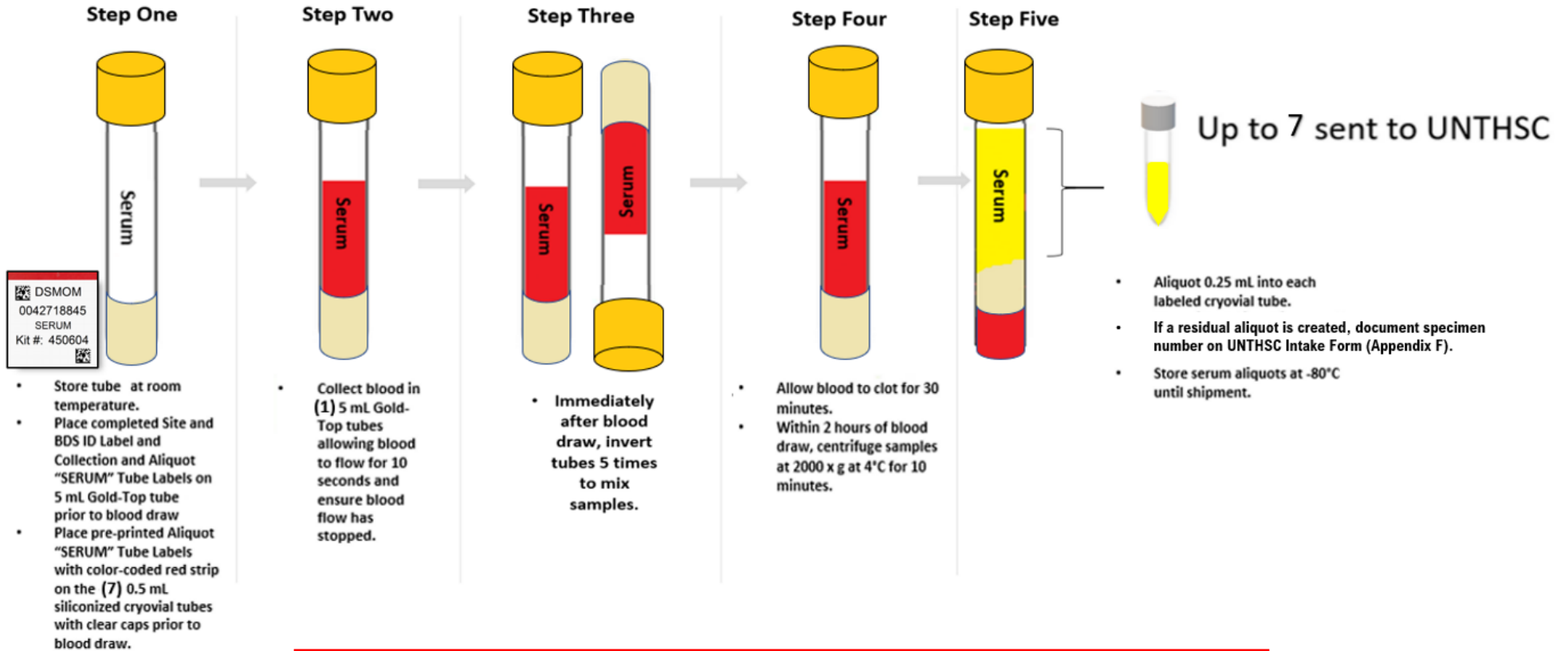
document the sample number and volume on the UNTHSC Intake Form  
([Appendix F](#)).



Close up view of 0.5 mL Cryovial

- Place 7 of labeled cryovials in the 25-cell cryovial box for UNTHSC. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to UNTHSC on dry ice.
- Dispose of collection tube with gel matrix and red blood cells at the bottom of the tube according to your site's guidelines for disposing of biomedical waste.

# Serum Preparation (5mL Gold-Top Tube) x 1



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

### 13.5 EDTA (Lavender-Top) Blood Collection Tube (10 mL) for Plasma and Buffy Coat x 1

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

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

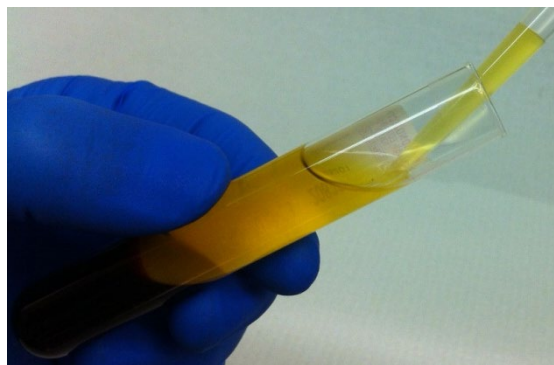
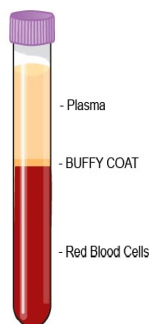
1. Set centrifuge to 4°C to pre-chill before use.
2. Place completed Site and BDS ID Label and pre-printed “**PLASMA**” Collection and Aliquot Tube Label on the lavender-top EDTA tube (1 x 10 mL). Place pre-printed Aliquot “**PLASMA**” Tube Labels with color-coded purple strip on the (17) 0.5 mL Cryovial tubes with clear caps. Place pre-printed “**BUFFY COAT**” Collection and Aliquot Tube Label on the (1) 2 mL cryovial with a blue lid.
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) x 1** 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 ‘UNTHSC Intake Form’. Do not attempt to draw an additional EDTA at this time. Process blood obtained in existing EDTA tube.
  6. **CRITICAL STEP:** Immediately after blood collection, **gently invert/mix (180 degree turns) the EDTA tubes 8-10 times.**



7. **CRITICAL STEP: Immediately after inverting the EDTA tubes, place it on wet ice until centrifugation begins.**
  - a. **If field-draw**, keep the samples on wet ice until you reach your destination. Please make note on [Appendix F](#) if field draw.
    - Preferably within 30 minutes of blood collection, centrifuge balanced tube for 10 minutes at 2000 RCF (x g) at 4°C. **It is critical that the tube is centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in [Appendix A](#) to calculate RPM. Equivalent rpm for spin at 2000 x g.**
    - Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection. **If field-draw**, place tube on wet ice until you reach the lab for processing.
  
8. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall, being careful not to agitate the buffy coat and packed red blood cells at the bottom of the tube (see below). Transfer plasma from the 10mL EDTA tube into the pre-labeled clear-cap “PLASMA” cryovials with color-coded purple strip. Aliquot 0.25 mL per cryovial (total vials = 17 with 0.25 mL each). The EDTA tube should yield, on average, 5 mL of plasma for a total of 17 0.5 mL Cryovial tubes per subject with 0.25 mL per cryovial tube. Be sure to only place **plasma** in Cryovials labeled with the “PLASMA” label with color-coded purple strip. Take caution not to disturb the red blood cells at the bottom of the tube. If there is extra plasma left, use 1 extra cryovial provided for another <0.25 mL aliquot of plasma. **If a residual aliquot (<0.25 mL) is created, document the sample number and volume on the UNTHSC Intake Form ([Appendix F](#)).**



**NOTE: When pipetting plasma from the plasma tube into 0.5mL Cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.**

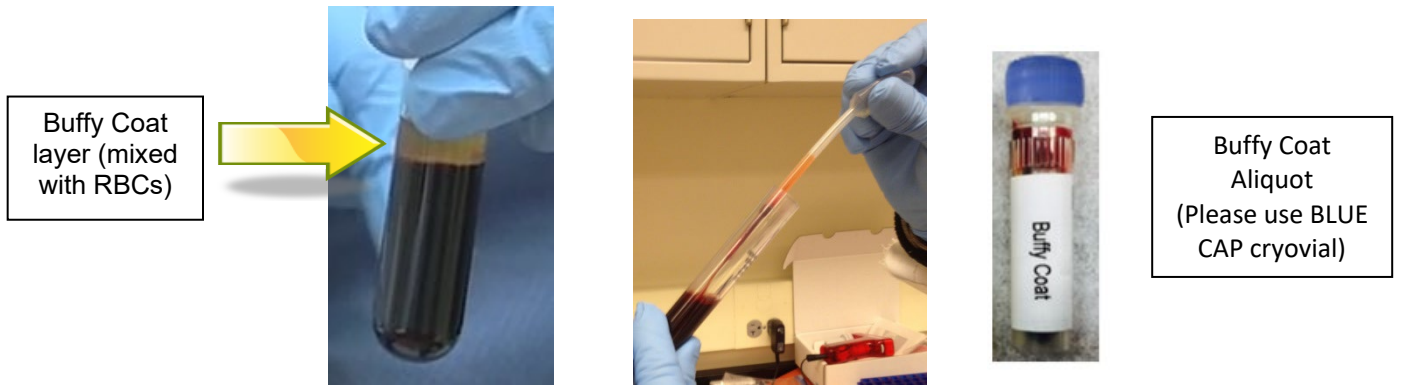
Plasma Aliquots  
(up to 17 possible)



Close up view of 0.5 mL  
Cryovial

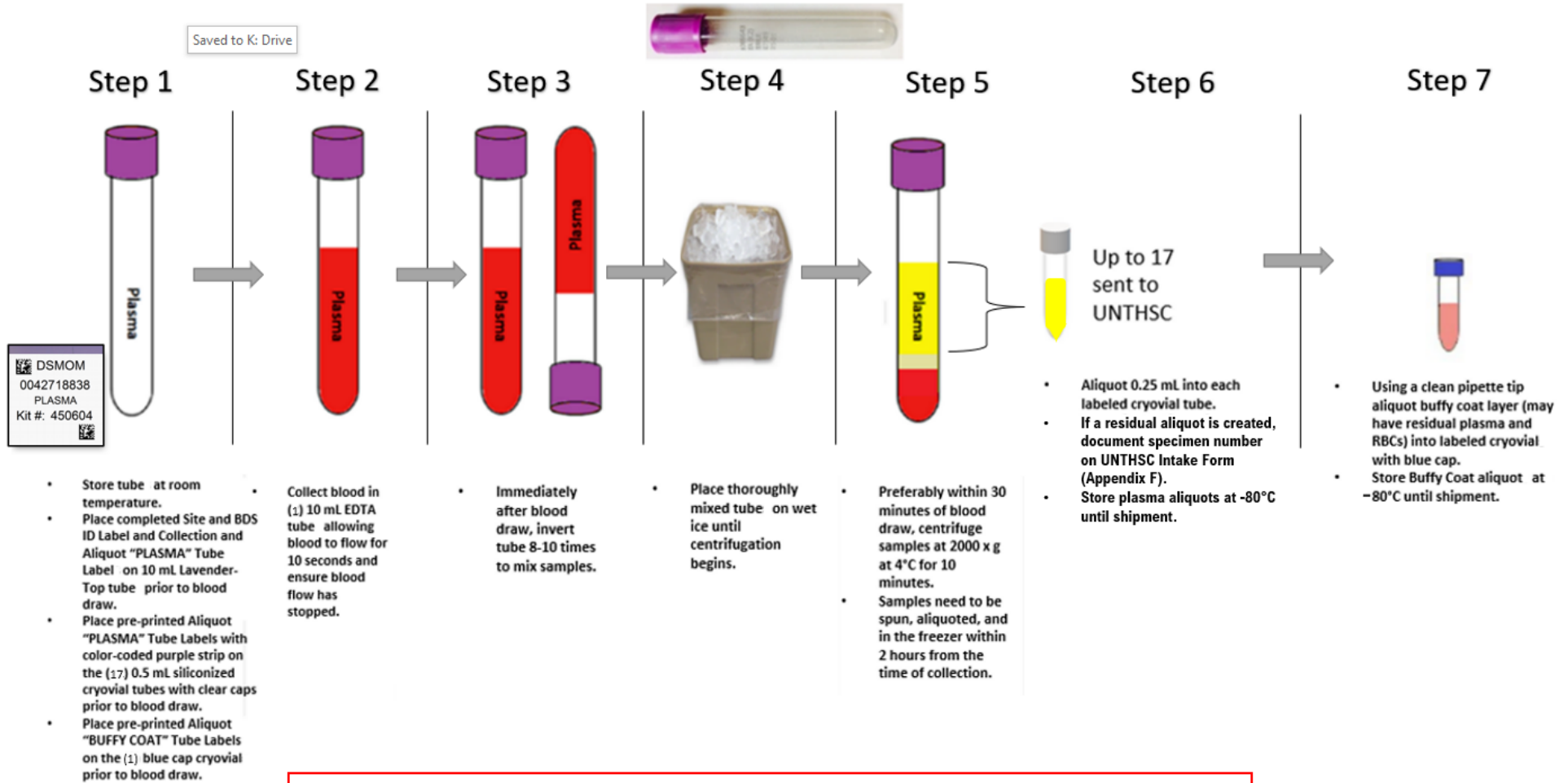


9. Place 17 of the labeled cryovials in the 25-cell cryovial box for UNTHSC and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to UNTHSC on dry ice.
10. After plasma has been removed from the EDTA (Lavender-Top) Blood Collection Tubes (10 mL), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) into labeled cryovial with blue cap using a micropipette. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovial with the blue cap and "BUFFY COAT" label.



11. Dispose of tubes with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.
12. Place the labeled buffy coat cryovial in the 25-cell cryovial box for UNTHSC and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to UNTHSC on dry ice.

# Plasma and Buffy Coat Preparation (10 mL Lavender-Top Tube) x1



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

#### 14.0 PACKAGING AND SHIPPING INSTRUCTIONS

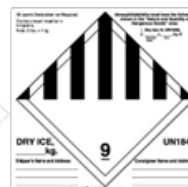
**ALL** study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your University, please contact NCRAD with questions and information regarding resources.

Sample Type	Processing/ Aliquoting	Tubes to UNTHSC	Ship
<b>Whole blood for isolation of serum</b>	0.25 mL serum aliquot per 0.5 mL Cryovial (clear cap)	7	Frozen
<b>Whole blood for isolation of plasma &amp; buffy coat (for DNA extraction)</b>	0.25 mL plasma aliquot per 0.5 mL Cryovial (clear cap)	17	Frozen
	1 mL buffy coat aliquot per 2.0 mL cryovial (BLUE CAP)	1	

**IMPORTANT!**  
**FROZEN SAMPLES MUST 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



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.

#### 14.1 Frozen Shipping Instructions

1. Contact FedEx (if UW-Madison, contact UPS) to confirm service is available and schedule package to be picked up.
2. On the day of scheduled pick-up, begin packaging specimens on dry ice **at least 1 hour before FedEx/UPS arrives**. Hold samples in -80°C freezer until it is time to package the specimens on dry ice for shipment.
3. Notify UNTHSC of shipment by emailing UNTHSC Lab Manager at: [Tori.Como@unthsc.edu](mailto:Tori.Como@unthsc.edu)
  - Attach the following to the email:
    - Completed UNTHSC Intake Form ([Appendix F](#) – also found on the [NCRAD ABC-DS study page](#)) and the UNTHSC Import Batch Form ([Appendix G](#)):
      - Aliquot barcodes need to be listed on the UNTHSC Import Batch Form ([Appendix G](#)). NCRAD will send an Excel file with all aliquot barcodes included in each kit when kit supplies are shipped.
    - If email is unavailable please call UNTHSC and do not ship until you've contacted and notified UNTHSC Lab Manager about the shipment in advance.
    - Please include the tracking number in the body of the email.
4. Place all frozen labeled aliquots of serum, plasma and buffy coat in the cryovial cryoboxes.
  - i. **FOR UNTHSC:** Each cryobox holds 25 samples and there will be approximately 25 cryovial samples being shipped to UNTHSC. Please place serum, plasma and buffy coat aliquots within one cryobox (7 serum, 17 plasma, 1 buffy coat) per participant blood draw (see below).



TO UNTHSC: 25-cell cryobox to contain  
Serum, Plasma, and Buffy Coat aliquots



- ii. Cryoboxes should contain all of the specimens from the same patient, per time point.
  - iii. **FOR UNTHSC – Batch shipping should be performed every 3 months or when specimens from 5 participants accumulate, whichever is sooner.**
5. Label the outside of the cryoboxes with the kit number label.
    - i. **FOR UNTHSC – Place serum, plasma, and buffy coat aliquots within one cryobox in a small biohazard bag.**
  6. Place the cryoboxes in the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag.

TO UNTHSC: 25-cell cryobox to contain Serum, Plasma and Buffy Coat aliquots



Place kit number label on each cryobox

7. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam shipping container.
8. Place the biohazard bag into the provided Styrofoam-lined shipping container on top of the dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container (as pictured below).
9. Fully cover the cryoboxes with approximately 2 inches of dry ice.

Full Shipping Container with Batched Samples and Dry Ice



10. 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.
11. Replace the lid on the Styrofoam carton. Place the completed UNTHSC Intake form and UNTHSC Import Batch form for UNTHSC in the package on top of the Styrofoam lid for each patient specimen. Close and seal the outer cardboard shipping carton with packing tape.
12. Complete the FedEx return airbill (if UW-Madison, follow UPS instructions provided at site) with the following information:
  - a. Section 1, "From": fill in your name, address, phone number, and Site FedEx Account Number.
  - b. Section 2, "Your Internal Billing Reference": add any additional information required by your site.
  - c. Section 6, "Special Handling and Delivery Signature Options": under "Does this shipment contain dangerous goods?" check the boxes for "Yes, Shipper's Declaration not required" and "Dry Ice". Enter the number of packages (1) x the net weight of dry ice in kg.
  - d. Section 7, "Payment", check sender and bill transportation costs to your site's study FedEx account number.
13. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
  - a. Your name and return address
  - b. Net weight of dry ice in kg (must match amount on the airbill)
  - c. Consignee name and address:

**UNTHSC**  
ATTN: Tori Conger  
3420 Darcy Street  
Fort Worth, TX 76107  
Phone: 817-735-2638
  - d. Do not cover any part of this label with other stickers, including pre-printed address labels.
14. Apply all provided warning labels and the completed FedEx return airbill to the outside of package, taking care not to overlap labels.



**IMPORTANT!**

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

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

**UNTHSC**

ATTN: Tori Conger  
3420 Darcy Street  
Fort Worth, TX 76107  
Phone: 817-735-2638

16. Use FedEx tracking to ensure the delivery occurs as scheduled and is received by UNTHSC. Please notify UNTHSC Lab Manager by email ([Tori.Como@unthsc.edu](mailto:Tori.Como@unthsc.edu)) that a shipment has been sent and include the FedEx tracking number in your email.

**\*\*\*Important Note\*\*\***

**For frozen shipments, include no more than five cryovial boxes (separated by patient within 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 UNTHSC as outlined above.**

- **Remember to complete the requisition forms and include a copy in your shipment: UNTHSC Intake Form ([Appendix F](#)) and UNTHSC Import Batch Form ([Appendix G](#)) for UNTHSC.**
- **Notify the UNTHSC contact by email at [Tori.Como@unthsc.edu](mailto:Tori.Como@unthsc.edu) IN ADVANCE to confirm the shipment (include FedEx tracking number in 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**

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



## 15.0 APPENDICES

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

[Appendix F: UNTHSC Intake Form](#)

[Appendix G: UNTHSC Import Batch Form](#)