



**ADDS MANUAL OF PROCEDURES UPDATE:**

**V03.2020**

<b>Section</b>	<b>Change</b>
Document Footer	The version date was updated for this amendment
Title Page	Title page updated to reflect NCRAD name change
Throughout document	1. Updated fax number and room number throughout.

# ADDS

Alzheimer's Disease in Down Syndrome

---

## Alzheimer's Disease in Down Syndrome (ADDS)

in collaboration with

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

*Blood-Based Biospecimens*

*Manual of Procedures*

*Version 4.3*

*March, 2020*



## **TABLE OF CONTENTS**

<b>1.0</b>	<a href="#">Abbreviations</a> .....	4
<b>2.0</b>	<a href="#">Purpose</a> .....	6
<b>3.0</b>	<a href="#">NCRAD Information</a> .....	7
3.1	<a href="#">NCRAD Contacts</a>	
3.2	<a href="#">Hours of Operation</a>	
3.3	<a href="#">Holiday Schedules</a>	
3.4	<a href="#">Holiday Observations</a>	
<b>4.0</b>	<a href="#">NCRAD Laboratory Collection</a> .....	9
4.1	<a href="#">Site Required Equipment</a>	
4.2	<a href="#">Biospecimen Collection Schedules (Specimens Sent to NCRAD Only)</a>	
4.3	<a href="#">Biospecimen Collection Charts</a>	
<b>5.0</b>	<a href="#">Specimen Collection Kits, Shipping Kits, and Supplies</a> .....	12
5.1	<a href="#">Specimen Collection Kit Contents</a>	
5.2	<a href="#">Kit Supply to Study Sites</a>	
<b>6.0</b>	<a href="#">Blood Collection and Processing Procedures</a> .....	14
6.1	<a href="#">Labeling Samples</a>	
6.2	<a href="#">Video List</a>	
6.3	<a href="#">Filling Aliquot Tubes (Plasma and Serum)</a>	
6.4	<a href="#">Serum Separator (Gold-Top) Blood Collection Tubes (5 ml) for Serum</a>	
6.5	<a href="#">EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Plasma and Buffy Coat</a>	
<b>7.0</b>	<a href="#">Incomplete or Difficult Blood Draws</a> .....	29
<b>8.0</b>	<a href="#">Packaging and Shipping Instructions</a> .....	29
8.1	<a href="#">Frozen Shipment Instructions</a>	
<b>9.0</b>	<a href="#">Data Queries and Reconciliation</a> .....	36
<b>10.0</b>	<a href="#">Appendices List</a> .....	36
	<a href="#">Appendix A: Rate of Centrifuge Worksheet</a>	
	<a href="#">Appendix B: Biological Sample and Shipment Notification Form</a>	



## 1.0 ABBREVIATIONS

ABC-DS	Alzheimer's Biomarker Consortium – Down Syndrome
AD	Alzheimer's Disease
ADDS	Alzheimer's Disease in Down Syndrome
CDCC	Columbia Data Coordinating Center
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
DS	Down Syndrome
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
NiAD	Neurodegeneration in Aging Down Syndrome: A Longitudinal Study of Cognition and Biomarkers of Alzheimer's Disease
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
SST	Serum Separator Tube

## 2.0 PURPOSE

The collection of blood-based biofluids is an important part of the Alzheimer's Disease in Down Syndrome (ADDS) study. These samples will be used to:

- Identify blood-based and genetic biomarkers associated with the transition from normal aging to mild cognitive impairment to clinical dementia in individuals with DS
- Understand biomarker relationships and the pathways implicated in AD pathogenesis and develop a model for predicting risk.
- 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 ADDS study visits. It includes instructions for blood-based biospecimen submission to NCRAD located in Indianapolis at Indiana University.

*The following samples will be sent to NCRAD:*

- Serum
- Plasma
- Buffy Coat (DNA extraction)

*Additional samples collected but not shipped to NCRAD:*

- CSF (Please see the ABC-DS Lumbar Puncture Manual of Procedures for details)

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

These procedures are relevant to all study personnel responsible for processing blood specimens entering NCRAD.



### **3.0 NCRAD INFORMATION**

#### **3.1 NCRAD Contacts**

**Tatiana Foroud, PhD, NCRAD Leader**

Phone: 317-274-2218

**Kelley Faber, MS, CCRC, Project Manager**

Phone: 317-274-7360

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

**Colleen Mitchell, Laboratory Manager**

Phone: 317-278-9016

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

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

Phone: 1-800-526-2839

Fax: 317-278-1100

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

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

ADDS-NiAD Study Specific Webpage:

[https://www.ncrad.org/resource\\_adds\\_niad.html](https://www.ncrad.org/resource_adds_niad.html)

**Kristi Wilmes, MS, CCRP Study Coordinator**

Phone: 317-274-7546

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

#### **Sample Shipment Mailing address**

NCRAD

Indiana University School of Medicine

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

TK-342

Indianapolis, IN 46202

### 3.2 Hours of Operation

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

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

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

### 3.3 Holiday Schedules

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

### 3.4 Holiday Observations

Date	Holiday
January 1	New Year's Day
3 <sup>rd</sup> Monday in January	Martin Luther King, Jr Day
4 <sup>th</sup> Monday in May	Memorial Day
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 20th by e-mailing [alzstudy@iu.edu](mailto:alzstudy@iu.edu), so that they can arrange to have staff available to process incoming samples.

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:

- Centrifuge capable of  $\geq 2000$  rcf 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 (SPECIMENS SENT TO NCRAD ONLY)

**ADDS Blood Based Biomarker Collection Schedule for NCRAD:**

	Baseline (1A)	3-Month (1B)	16-Month (2A)	19-Month (2B)	32-Month (3A)	35-Month (3B)
Serum		X		X		X
DNA		X		X		X
Plasma		X		X		X

Whole blood is collected into two different types of tubes (gold top Serum Separator tubes and lavender top EDTA tube). These tubes are processed locally into serum, plasma, and buffy coat fractions; they are then aliquoted, frozen at the study site, and shipped to NCRAD.

Consent forms must specify that any biological samples and de-identified clinical data may be shared with academic and/or industry collaborators through





NCRAD. A copy of the consent form for each subject should be kept on file by the site investigator.

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

### 4.3 BIOSPECIMEN COLLECTION CHART

#### 4.3.1 Blood Collection

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD
Whole blood for isolation of serum	Serum Separator (Gold-Top) Blood Collection Tube (5 ml)	2	N/A	N/A
	SERUM: 0.5 ml cyrovials	21	0.25 ml serum aliquot per 0.5 ml siliconized cyrovial (clear cap with RED sticker)	16-21
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	EDTA (Lavender-Top) Blood Collection Tube (10 ml)	1	N/A	N/A
	PLASMA: 0.5 ml cyrovials	21	0.25 ml plasma aliquot per 0.5 ml siliconized cryovial (clear cap with LAVENDER sticker)	16-21
	BUFFY COAT: 2.0 ml cyrovial	1	1 ml buffy coat aliquot per 2.0 ml cyrovial (BLUE CAP)	1

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

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

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 plasma/buffy coat/serum/CSF aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, Site and Subject 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.*

#### ADDS Blood Kit

Quantity	ADDS Blood Based Kit Components
1	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
2	Serum Separator (Gold-Top) Blood Collection Tube (5 ml)
1	15 ml conical
21	Siliconized cryovial tube (0.5 ml) with clear cap with lavender sticker
21	Siliconized cryovial tube (0.5 ml) with clear cap with red sticker
1	Cryovial tube (2.0 ml) with blue cap
1	Disposable graduated transfer pipette
46	Pre-printed Collection and Aliquot Tube Label
4	Pre-printed Kit Number Label
4	Labels for handwritten Site and Subject ID
2	Microcentrifuge tube box (holds up to 25 microcryovials)



**ADDS Frozen Blood Shipping Supply Kit**

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

**Blood Supplemental Supply Kit**

Quantity	Blood Supplemental Supply Kit Components
10	Siliconized cryovial tube (0.5 ml) with clear cap with lavender sticker
10	Siliconized cryovial tube (0.5 ml) with clear cap with red sticker
5	Cryovial tube (2.0 ml) with blue cap
5	15 ml conical
5	Microcentrifuge tube box (holds up to 25 microcryovials)
5	Disposable graduated transfer pipette
5	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
10	Serum Separator (Gold-Top) Blood Collection Tube (5 ml)
10	Labels for handwritten Site and Subject ID

**Individual Supplies**

Available upon request within the NCRAD kit module.

Quantities	Item
By Request	25 cell cryobox
By Request	Siliconized Cryovial tube (0.5) with clear cap with lavender sticker
By Request	Siliconized Cryovial tube (0.5) with clear cap with red sticker
By Request	Cryovial tube (2 ml) with blue cap
By Request	FedEx return airbill
By Request	15 ml conical tube
By Request	Small IATA shipping box for ambient shipping
By Request	Shipping container for dry ice shipment (shipping and Styrofoam box)
By Request	Plastic biohazard bag
By Request	Disposable graduated transfer pipette
By Request	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
By Request	Serum Separator (Gold-Top) Blood Collection Tube (5 ml)

By Request	Warning label packet
By Request	Fine Point permanent markers
By Request	Site and Subject ID Labels

## 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 [ADDS-NiAD 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 weeks** for kit orders to be processed and delivered.

## 6.0 BLOOD COLLECTION AND PROCESSING PROCEDURES

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

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

- 1. Serum Separator (Gold-Top) Blood Collection Tube (5 ml) for Serum x 2**
- 2. EDTA (Lavender-Top) Blood Collection Tube (10 ml) for DNA and Plasma**

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

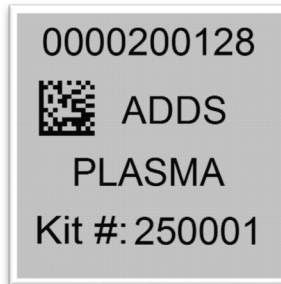
### 6.1 Labeling Samples

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

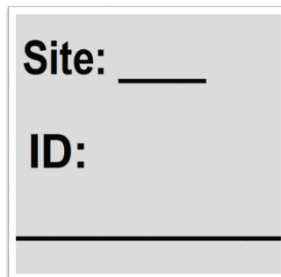
1. Kit Number Label
2. Collection and Aliquot Tube Label
3. Site and Subject ID Label



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



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



The **Site and Subject ID Labels** are placed on all collection tubes, both blood and CSF.

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

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

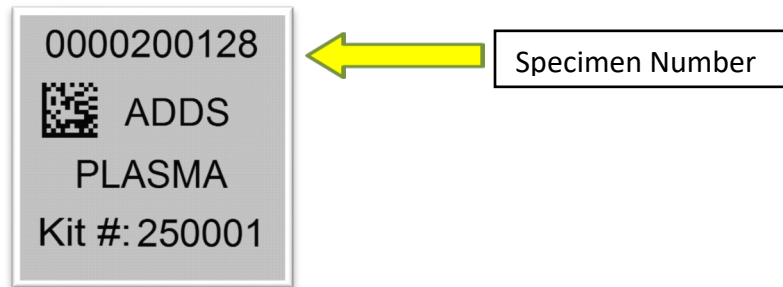


**Serum Separator (Gold-Top)  
Blood Collection Tube (5 ml)**

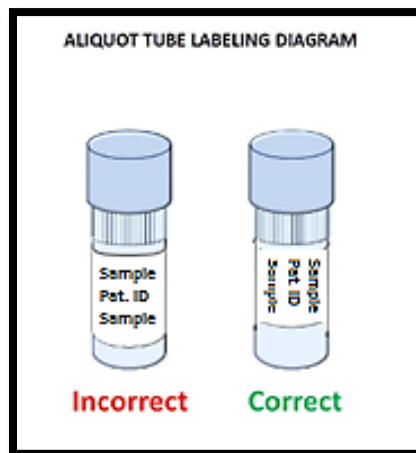
**EDTA (Lavender-Top) Blood  
Collection Tube (10 ml)**

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 at the top of the label. This ensures that no aliquot is misplaced or lost during the shipment process. (see Plasma Collection and Aliquot Tube Label on next page)



- Using a fine point permanent marker, fill-in and place the Site and Subject ID Labels on the collection tubes only (EDTA and SST) **BEFORE** sample collection, processing, or freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place Site and Subject ID labels on any cryovials.
- The Collection and Aliquot Tube Labels contain a 2D barcode on the left hand side of the label. Place this barcode toward the tube cap.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see attached labeling diagram).
- Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label.



- 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.

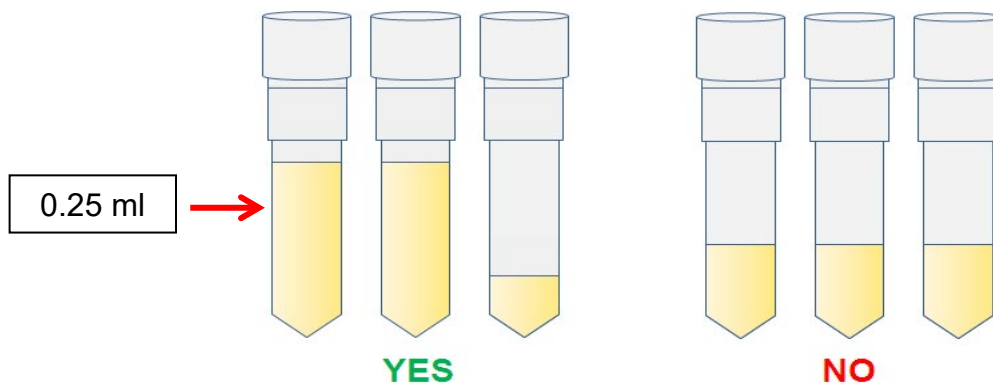


## 6.2 Video List

- The following training videos are available to assist you with the specimen processing, aliquoting, and shipping processes. The videos are available at: [https://ncrad.org/resource\\_adds\\_niad.html](https://ncrad.org/resource_adds_niad.html).
  - ADDS MOP Training
  - Plasma and Buffy Coat Processing and Aliquoting
  - Serum Processing and Aliquoting
  - Frozen Shipping

## 6.3 Filling Aliquot Tubes (Plasma and Serum)

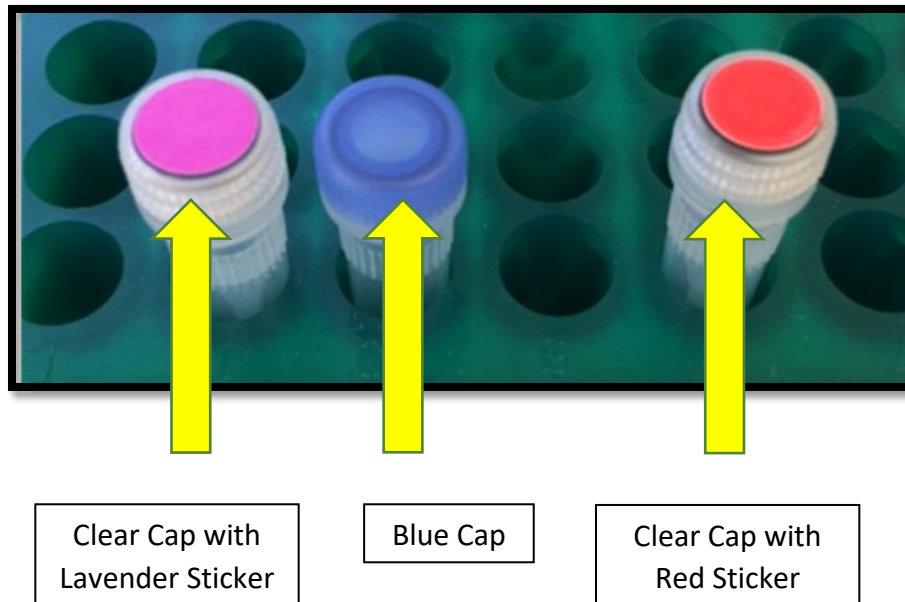
In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each cryovial should be filled to 0.25 milliliters (see picture below) with the respective biological material after processing is completed (refer to 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. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. 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.

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

Cap Color	Sample Type
Clear Cap with Lavender Sticker	Plasma
Blue Cap	Buffy Coat
Clear Cap with Red Sticker	Serum



#### 6.4 Serum Separator (Gold-Top) Blood Collection Tube (5 ml) for Serum

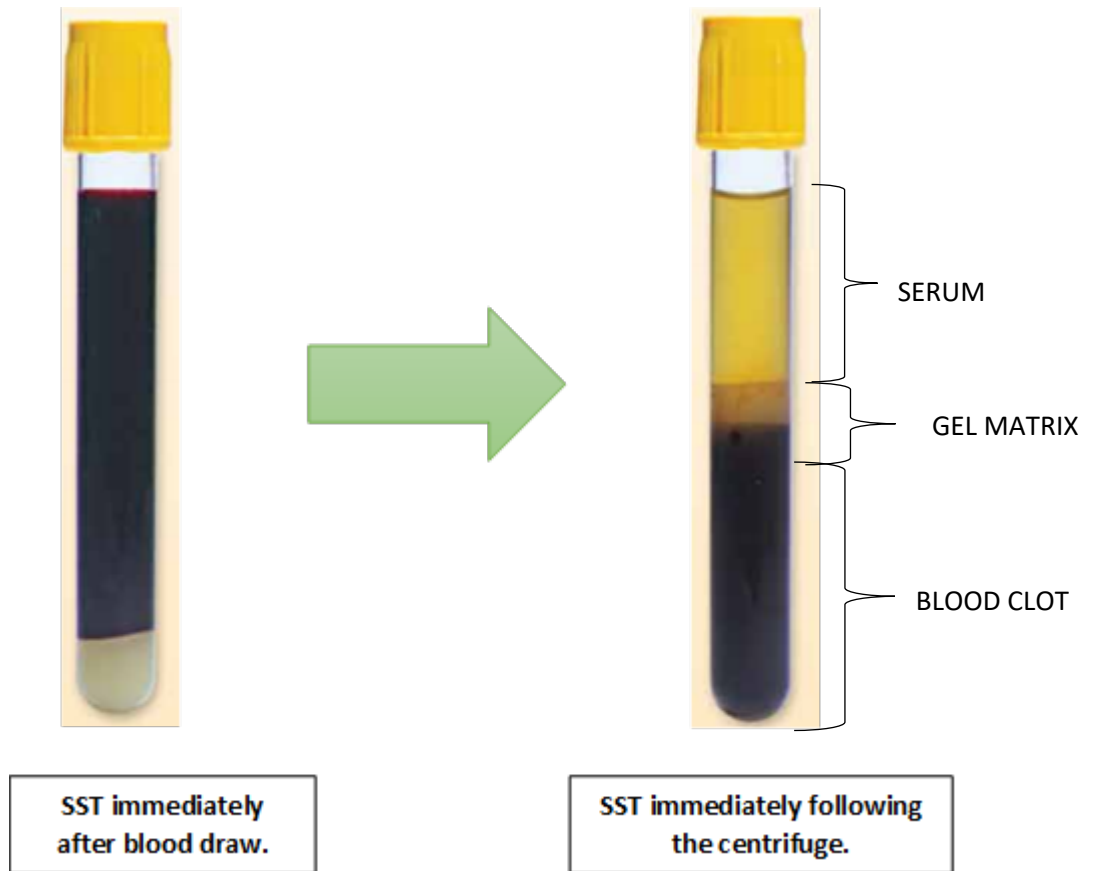
**Whole Blood Collection for Isolation of Serum: Serum Separator (Gold-Top) Blood Collection Tube (5 ml) (for processing of serum aliquots).**

1. Set centrifuge to 4°C to pre-chill before use.
2. Place completed Site and ADDS 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 Collection and Aliquot **“SERUM”** Tube Labels on the (21) 0.5 ml siliconized cryovial tubes with clear caps and red stickers.

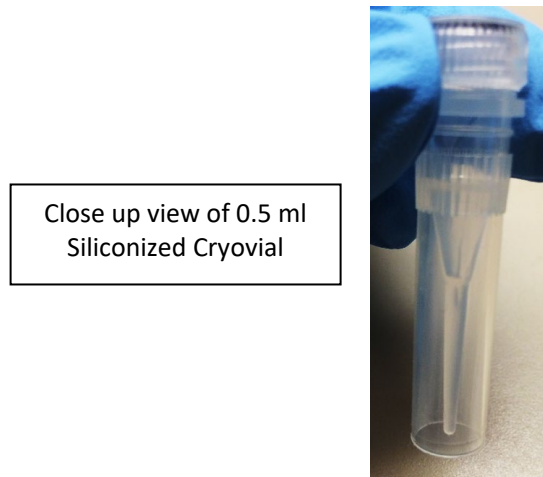
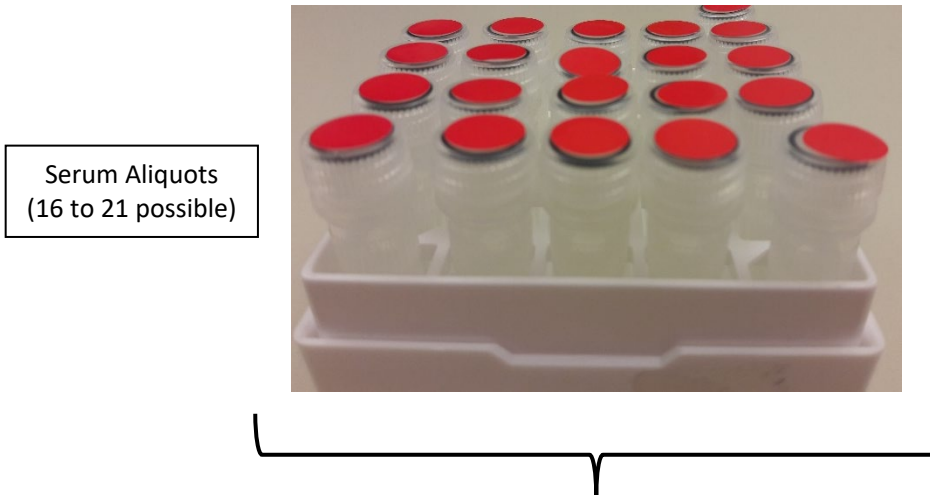
3. Using a blood collection set and a holder, collect blood into **Serum Separator (Gold-Top) Blood Collection Tubes (2 x 5 ml)** using your institution's recommended procedure for standard venipuncture technique

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

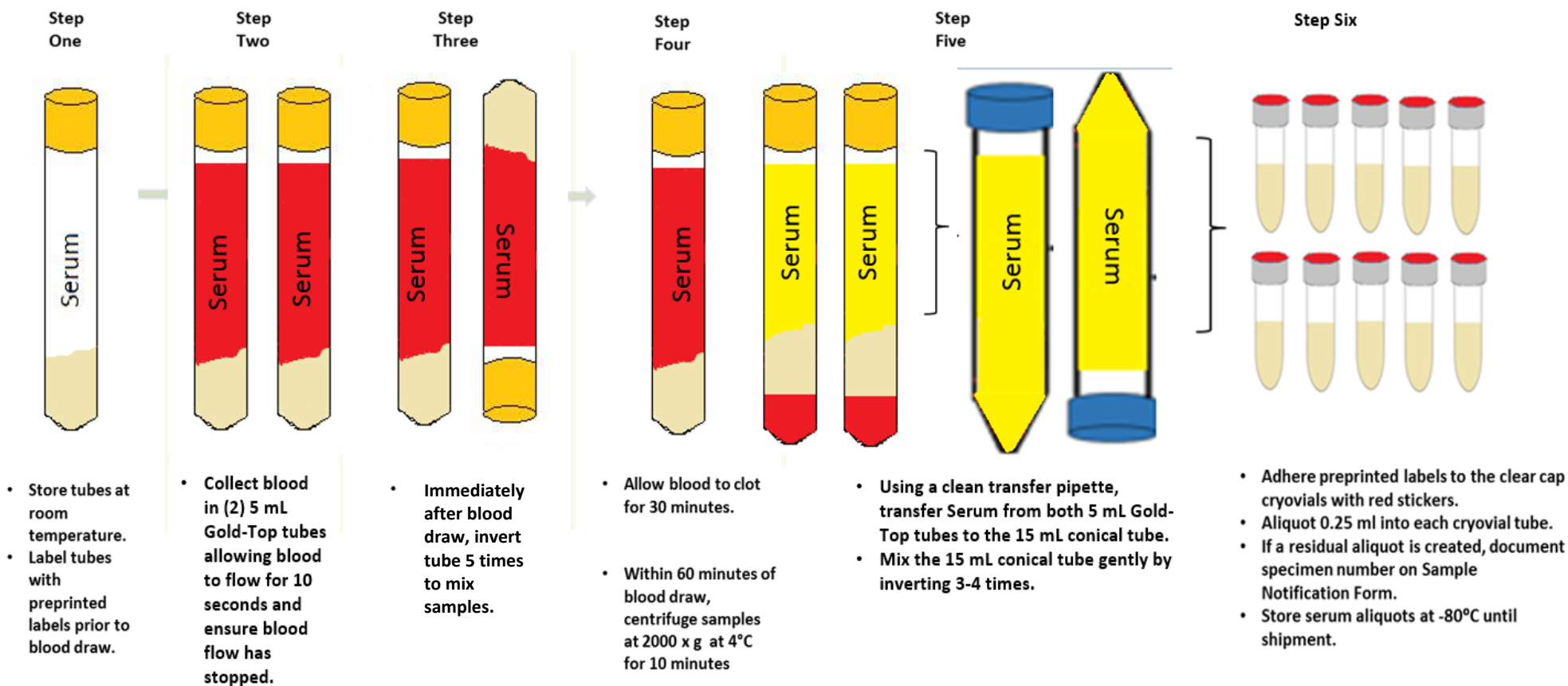


8. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the wall. Transfer serum from both gold top Serum Separator tubes into the single 15ml conical tube. Mix the serum by gently inverting the conical tube 3-4 times.
9. Using a pipette, transfer serum from the 15 ml conical tube, into the pre-labeled siliconized cryovials with the red stickers on the caps. 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 siliconized cryovials labeled with the "SERUM" label and red stickers on the caps. 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.**



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



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

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

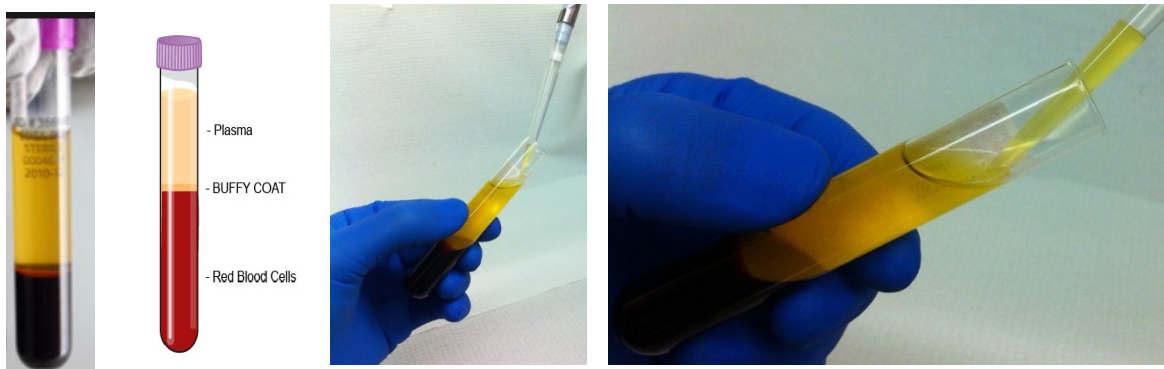
1. Set centrifuge to 4°C to pre-chill before use.
2. Place completed Site and Subject ID Label and pre-printed “**PLASMA**” Collection and Aliquot Tube Label on the lavender-top EDTA tube. Place pre-printed “**PLASMA**” Collection and Aliquot Tube Labels on the (21) 0.5 ml siliconized cryovial tubes with clear caps and lavender stickers. 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)** using your institution's recommended procedure for standard venipuncture technique.

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

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



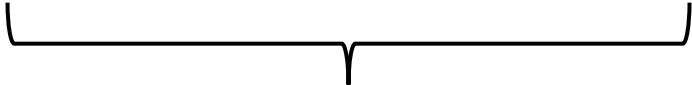
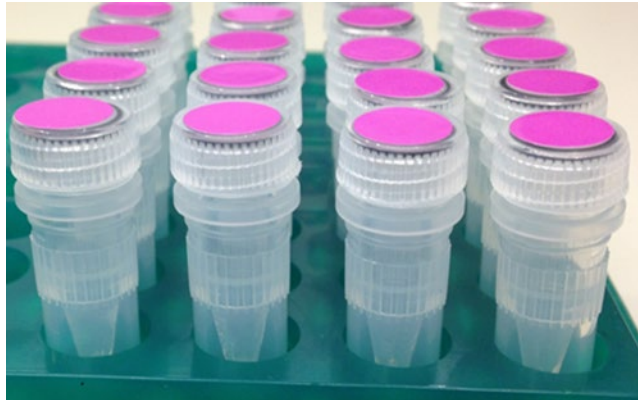
7. **CRITICAL STEP: Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.**
  - Preferably within 30 minutes of blood collection, centrifuge balanced tubes for 10 minutes at 2000 RCF (x g) at 4°C. **It is critical that the tubes be centrifuged at the appropriate 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.
  - Record time aliquoted on the Biological Sample and Shipment Notification Form.
  
8. Remove the plasma, being careful not to agitate the packed red blood cells at the bottom of the tube. Tilt the tube and placing the disposable pipette tip along the lower side of the wall without touching the pellet (buffy coat) so that plasma is not contaminated (see below). Transfer plasma into the pre-labeled cryovials. Aliquot 0.25 ml per cryovial (total vials = 16-21 with 0.25 ml each). The EDTA tube should yield, on average, 5 ml of plasma for a total of 16-21 0.5 ml siliconized cryovial tubes per subject with 0.25 ml per cryovial tube. Be sure to only place **plasma** in cryovials labeled with “PLASMA” labels. Take caution not to disturb the red blood cells at the bottom of the tube. If there is extra plasma left, use 1 extra cryovial 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 cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.**

Plasma Aliquots  
(16 to 21 possible)

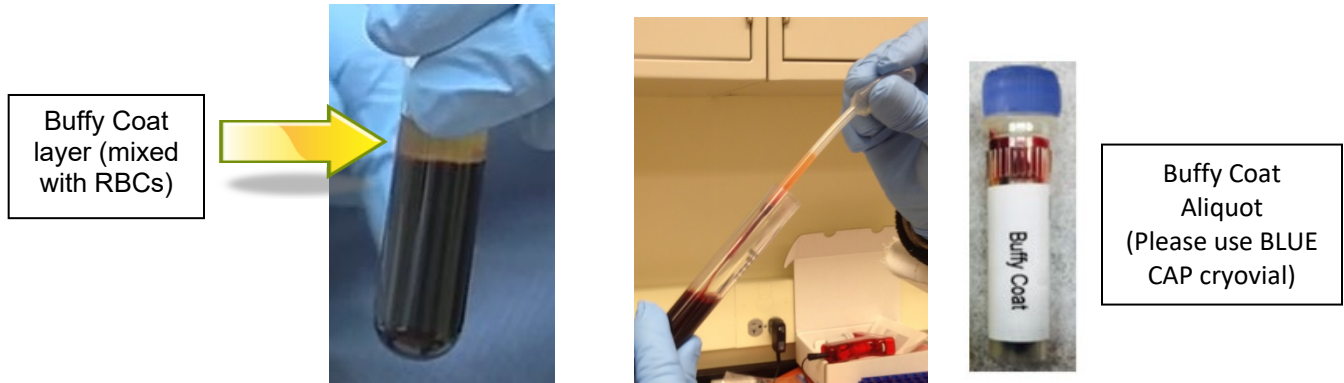


Close up view of 0.5 ml  
Siliconized Cryovial



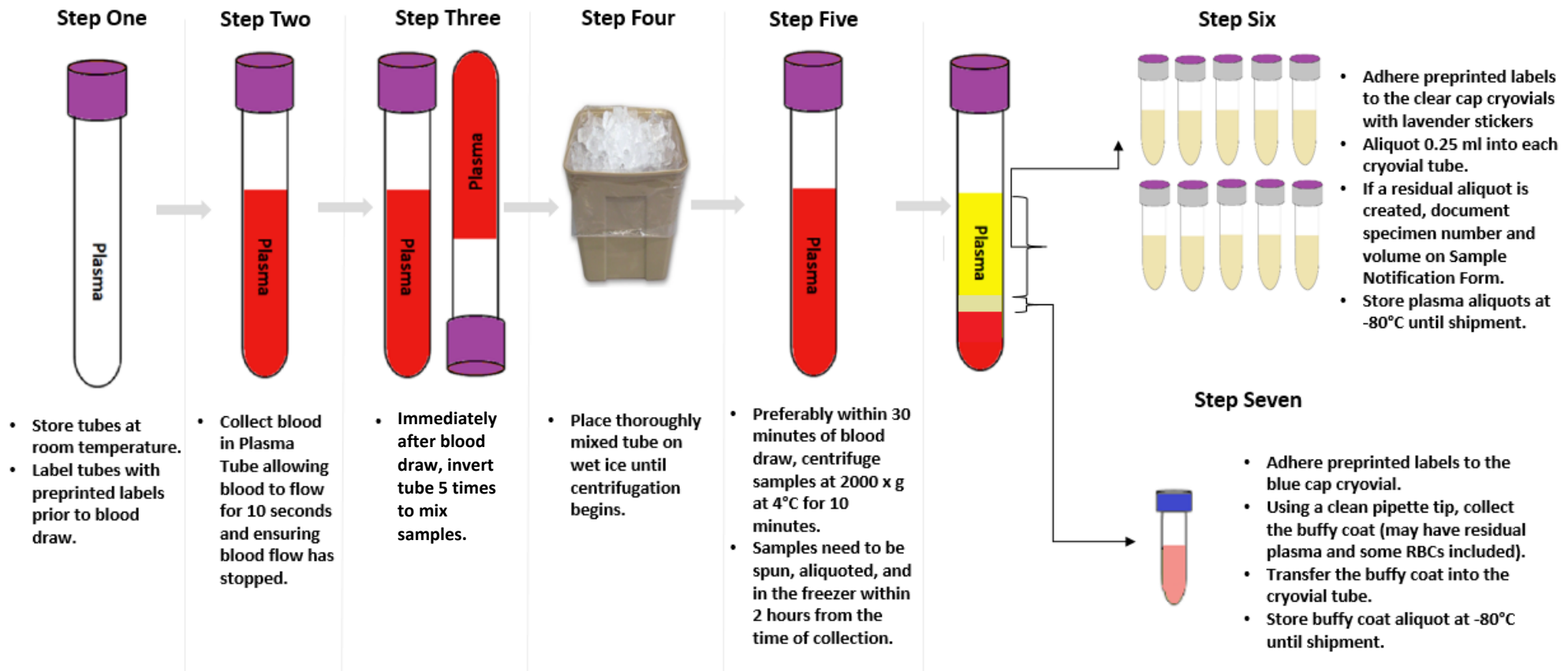
9. Place the labeled cryovials in the 25 cryovial box and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification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

10. After plasma has been removed from the EDTA (Lavender-Top) Blood Collection Tube (10 ml), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) into labeled cryovial with blue cap using a disposable graduated micropipette. All of the buffy coat will be placed into one cryovial. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovial with the blue cap and “BUFFY COAT” label.



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

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



## 7.0 INCOMPLETE OR DIFFICULT BLOOD DRAWS

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

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

**Redraws will not be scheduled for samples submitted to NCRAD as participants are seen longitudinally.**

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 NCRAD
    - i. Indicate blood draw difficulties at the bottom of the 'Biological Sample and Shipment Notification Form' within the "Notes" section.
    - ii. Complete the 'Biological Sample and Shipment Notification Form' with tube volume approximations and number of aliquots created.
  - c. Contact a NCRAD coordinator and alert them of the challenging blood draw
2. *If the biospecimens at a scheduled visit **are not** collected:*
  - a. Contact the ADDS Global Coordinator 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.

## 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	ADDS	Processing/ Aliquoting	Tubes to NCRAD	Ship
<b>Whole blood (Gold-Top SST) for isolation of serum</b>	<b>Yes</b>	0.25 ml serum aliquots per 0.5 ml siliconized cryovial (clear caps with RED stickers)	16-21	Frozen
<b>Whole blood (Lavender-Top EDTA) for isolation of plasma &amp; buffy coat (for DNA extraction)</b>	<b>Yes</b>	0.25 ml plasma aliquots per 0.5 ml siliconized cryovial (clear caps with LAVENDER stickers)	16-21	Frozen
	<b>Yes</b>	1 ml buffy coat aliquot per 2.0 ml cryovial (BLUE cap)	1	Frozen

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

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

**\*\*\* 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

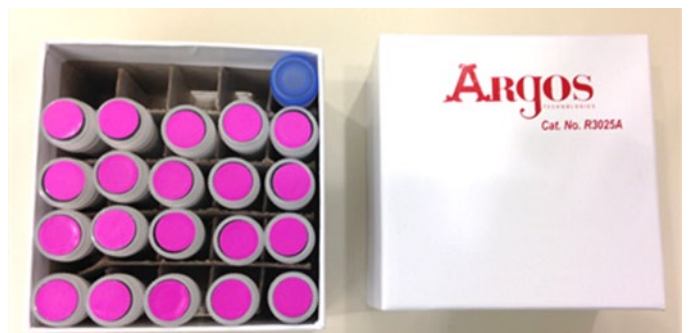
1. Contact FedEx to confirm service is available and schedule package to be picked up.
2. Notify NCRAD of shipment by emailing NCRAD coordinators at: [alzstudy@iu.edu](mailto:alzstudy@iu.edu)

Attach the following to the email:

- Completed Biological Sample and Shipment Notification Form to the email notification.  
(See [Appendix B](#) for an example of the NCRAD sample form)
  - If email is unavailable please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.
3. Place all frozen labeled aliquots of plasma and buffy coat in the cryovial cryobox.
    - i. Each cryobox holds 25 samples and there will be approximately 40 cryovial samples. Please place plasma and buffy coat aliquots within one cryobox and the serum aliquots within the second cryobox. (21 plasma, 21 serum, and 1 buffy coat) per blood draw (see below).



One cryobox to contain Serum Aliquots.



One cryobox to contain Plasma and Buffy Coat Aliquots.

- ii. Cryoboxes should contain all of the specimens from the same patient, per time point.
- iii. **Batch shipping should be performed every 3 months or when specimens from 5 participants accumulates, whichever is sooner.**



4. Label the outside of the cryoboxes with the kit number label. Place Plasma/Buffy Coat aliquots within one cryobox and the serum aliquots in another cryobox. BOTH cryoboxes should be placed within the SAME biohazard bag. These biohazard bags are large enough to contain one or two cryoboxes.
5. 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.



Place kit  
number label on  
each cryobox

Plasma, Buffy Coat, and Serum Cryoboxes placed in clear biohazard bag

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

Full Shipping Container with  
Batched Samples and Dry Ice







9. Replace the lid on the Styrofoam carton. Place the completed Biological Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape.
  
10. Complete the FedEx return airbill with the following information:
  - a. Section 1, “From”: fill in your name, address, phone number, and Site FedEx Account Number.
  - b. Section 2, “Your Internal Billing Reference”: add any additional information required by your site.
  - c. Section 6, “Special Handling and Delivery Signature Options”: under “Does this shipment contain dangerous goods?” check the boxes for “Yes, Shipper’s Declaration not required” and “Dry Ice”. Enter the number of packages (1) x the net weight of dry ice in kg.
  - d. Section 7, “Payment”, check third party and bill transportation costs to the ADDS study FedEx account number.
  
11. 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:

NCRAD  
IU School of Medicine  
351 West 10<sup>th</sup> Street  
TK-342  
Indianapolis, IN 46202
  - d. Do not cover any part of this label with other stickers, including pre-printed address labels.
  
12. Apply all provided warning labels and the completed FedEx return airbill to the outside of 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.**

13. Hold packaged samples in -80°C freezer until time of FedEx pick-up/drop-off.
  
14. 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  
Walther Hall – R3-C102  
351 West 10<sup>th</sup> Street  
TK-342  
Phone: 1-800-526-2839

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

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

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

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

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

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

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

## **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 Columbia Data Coordinating Center (CDCC) 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 CDCC database will be queried through the standard system. Additional discrepancies that may be unrelated to data entry will be resolved with the Principal Investigator in a separate follow up communication. If applicable, a non-conformance report will be provided to sites on a monthly basis.

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

- Missing samples
- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Biological Sample and Shipment Notification Form and logged at NCRAD compared to information entered into the DCC 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 A Rate of Centrifuge Worksheet

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

### Submitter Information

Name:

Site:

Submitter e-mail:

### Centrifuge Information

Please answer the following questions about your centrifuge.

#### Centrifuge Type

Fixed Angle Rotor:

Swing Bucket Rotor:

#### Radius of Rotation (mm):

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

#### Calculating RPM from G-Force:

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

RCF = Relative Centrifugal Force (G-Force)

RPM = Rotational Speed (revolutions per minute)

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

Comments:

**Please send this form to NCRAD Study Coordinator**

**317-278-1100 (Fax)**

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



**Appendix B**



Subject ID: \_\_\_\_\_ Site ID: \_\_\_\_\_ MGH InitID: \_\_\_\_\_  
 Date: \_\_\_\_/\_\_\_\_/\_\_\_\_ Cycle Visit: 1A 1B 2A 2B 3A 3B

**Biological Sample and Shipment Notification Form**

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

To: Kelley Faber    Email: alzstudy@iu.edu    FAX: 317-321-2003    Phone: 1-800-526-2839	
<i>General Information:</i>	
From: _____	Date: _____
Phone: _____	Email: _____
Study: ADDS <input type="checkbox"/> DS Participant	Kit #: _____
Sex: <input type="checkbox"/> M <input type="checkbox"/> F      Year of Birth: _____	KIT BARCODE
FedEx tracking #: _____	
<i>Blood Collection:</i>	
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]
5. Was the EDTA tube placed on ice immediately after inverting tube 5 times until centrifugation began? <input type="checkbox"/> Yes <input type="checkbox"/> No	
<i>Blood Processing:</i>	
<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 (1x10 mL EDTA tube): _____ mL	Original volume drawn (2x5 mL Serum tube): _____ mL
• Time aliquoted: _____ [HHMM]	• Time aliquoted: _____ [HHMM]
Number of 0.25 mL plasma aliquots created (16-20 total) (Siliconized cryovial): _____ x 0.25 mL	Number of 0.25 mL serum aliquots created (16-20 total) (Siliconized cryovial): _____ x 0.25 mL
• 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 aliquot created (one per EDTA tube) (Blue cap cryovial): _____ mL	<i>Note: Bulleted items not entered into eCRF.</i>
Notes:	

Version 03.2020