V02.2020

Section	Change
Document Footer	The version date was updated for this amendment.
Throughout Document	Shipping address room number change to Rm. 217
Throughout Document/Appendix sample forms	Fax number changed to 317-321-2003

Parkinson's Spectrum Disorders Center

UCSF Weill Institute for Neurosciences

Manual of Procedures

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

Parkinson's Spectrum Disorder Center (PSDC)

Biospecimen Collection, Processing, and Shipment Manual

Version 06.2019

TABLE OF CONTENTS

1.0 Abbreviations

CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
IUGB	Indiana University Genetics Biobank
LP	Lumbar Puncture
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
PBMC	Peripheral Blood Mononuclear Cell
PSDC	Parkinson's Spectrum Disorder Center
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RNA	Ribonucleic Acid
RPM	Revolutions Per Minute

2.0 PURPOSE

The purpose of this manual is to provide PSDC staff (PIs, study coordinators, and the sample collection and processing teams) at the study site with instructions for collection and submission of biological samples for PSDC study visits. It includes instructions for biospecimen submission to the National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD) located at Indiana University. The following samples may be collected at each study visit:

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

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

These procedures are relevant to all study personnel responsible for processing blood and CSF specimens to be submitted to NCRAD for the PSDC protocol.

3.0 NCRAD INFORMATION

3.1 NCRAD Contacts

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

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

Madeline Potter, BA, CCRP, Study Coordinator
Phone: 317-278-9546
Email: mkpotter@iu.edu

General NCRAD Contact Information
Phone: 1-800-526-2839
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: www.ncrad.org
PSDC Study Specific Webpage: https://www.ncrad.org/resource_psdc.html

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

3.2 Hours of Operation

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

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

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

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

3.3 Holiday Schedules

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

3.4 Holiday Observations

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

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

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

4.0 PSDC LABORATORY COLLECTION

4.1 Site Required Equipment

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

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

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

- Centrifuge capable of ≥ 1500 rcf (1500 x g) with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:

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

4.2 Biospecimens Sent to NCRAD

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

	PSDC Visit 1
DNA (Buffy Coat)	X
Plasma	X
PBMC	X
Serum	X
RNA	X
CSF	X*

*Select participant populations only

Whole blood will be collected into four different types of collection tubes (lavender-top EDTA tube, green-top sodium heparin tube, red-top serum determination tube, and PAXgene™ tubes). The lavender-top EDTA tubes are processed locally into plasma and buffy coat fractions, aliquoted, frozen at the study site, and then shipped to NCRAD. The green-top Sodium Heparin tubes (for PBMCs) are kept ambient without further processing and shipped to NCRAD the same day of the blood draw. The red-top Serum Determination tube is processed locally into serum fractions, aliquoted, frozen at the study site, and then shipped to NCRAD. The PAXgene™ tubes are frozen locally without further processing and shipped to NCRAD.

CSF will be aliquoted locally, frozen at the study site, and then shipped to NCRAD.

Consent forms must specify that any biological samples and de-identified clinical data may be shared with academic and/or industry collaborators through NCRAD. A copy of the consent form for each 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 Charts

4.3.1 Blood Collection

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	**3**	**3**	**N/A**	**N/A**
	PLASMA: 2 ml cryovials with lavender caps (residual volume placed in 2 ml cryovial with blue cap)	11 (10 Lavender Cap, 1 Blue Cap Cryovial)	1.5 ml plasma aliquots per 2.0 ml cryovial	8-10	Frozen
	BUFFY COAT: 2 ml cryovial with a clear cap	3	1 ml buffy coat aliquot per 2.0 ml cryovial	3	Frozen
Whole blood for PBMC isolation	**Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)**	**2**	**N/A**	**2**	**Ambient**
Whole blood for isolation of serum	**Serum Determination (Red-Top) Blood Collection Tube (10 ml)**	**1**	**1**	**N/A**	**N/A**
	Serum: 2 ml cryovials with red caps (residual volume placed in 2.0 ml cryovial with blue cap)	4 (3 Red Cap, 1 Blue Cap Cryovial)	1.5 ml Serum Aliquots Per 2.0 ml cryovial	2-4	Frozen
Whole blood for RNA isolation	**PAXgene™ Blood Collection Tube (2.5 ml)**	**3**	**N/A**	**3**	**Frozen**

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

4.3.2 Cerebrospinal Fluid

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes to NCRAD	Ship
CSF	Individually Packaged Sterile 50ml Conical Tube with blue caps	**2**	**N/A**	**N/A**	**N/A**
	2 ml cryovial with orange cap (residual volume placed in 2 ml cryovials with blue caps)	17 (16 Clear Cap, 1 Blue Cap Cryovial)	1.5 ml CSF aliquots per 2 ml cryovials	12-16	frozen
	2 ml cryovial with yellow cap	1	1.0-2.0 ml CSF aliquots per 2 ml cryovials	0	To local lab

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

5.0 SPECIMEN COLLECTION KITS, SHIPPING KITS AND SUPPLIES

Research specimen collection kits as well as clinical lab supplies (except dry ice and equipment supplies listed above) will be provided by NCRAD. These materials include blood tubes, Lumbar Puncture trays (when applicable), boxes for plasma/buffy coat/CSF aliquots storage and shipment, as well as partially completed shipping labels to send materials to NCRAD. Barcoded kit labels, site and PIDN labels, collection tube labels, and aliquot tube labels will all be provided by NCRAD. Collection tube labels and aliquot tube labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 6.1.

5.1 Specimen Collection Kit Contents

Collection kits contain the following (for each 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.*

PSDC Blood Kit

Quantity	PSDC Blood Kit Components
3	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
2	Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)
1	Serum Determination (Red-Top) Blood Collection Tube (10 ml)
3	PAXgene™ Blood Collection Tube (2.5 ml)
1	15ml orange cap conical tube
10	Cryovial tube (2 ml) with lavender cap
3	Cryovial tube (2 ml) with red cap
3	Cryovial tube (2 ml) with clear cap
2	Cryovial tube (2 ml) with blue cap
27	Pre-printed labels for blood collection and aliquot tubes
5	Pre-printed labels with kit number
10	Labels for handwritten Site and PIDN
1	Cryovial tube box (holds up to 25 cryovials)
1	Shipping Supplies for ambient shipment of PBMCs: Plastic biohazard bag with absorbent sheet Small IATA shipping box with insulated cooler Small refrigerant pack Aqui-Pak 6 tube absorbent pouch UN3373 Biological Substance Category B label List of contents card FedEx return airbill and pouch FedEx Clinic Pak

Optional NCRAD Kit (CSF)

Quantity	NCRAD CSF Kit Components
16	Cryovial tube (2 ml) with orange cap
1	Cryovial tube (2 ml) with blue cap
1	Cryovial tube (2 ml) with yellow cap
1	Lumbar Puncture tray (24G)
2	Individually Packaged Sterile 50ml Conical Tube
1	Cryovial tube box (holds up to 25 cryovials)
17	Pre-printed labels for blood collection and aliquot tube
5	Pre-printed labels with kit number

Frozen Shipping Supply Kit

Quantity	Frozen Shipping Kit Components
4	Plastic Biohazard bag with 250 mL absorbent sheets
1	FedEx return airbill and pouch
1	Shipping box/Styrofoam container
1	Warning label packet with dry ice sticker
12	Bubble wrap pouch

Green Top-Sodium Heparin Tube Redraw/Take Home Kit

Quantity	Ambient Shipping Supply Components
2	Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)
2	Pre-printed label for blood collection tube
3	Label for handwritten Site and PIDN
2	Pre-printed labels with kit number
1	Shipping Supplies for ambient shipment of PBMCs: Plastic biohazard bag with absorbent sheet Small IATA shipping box with insulated cooler Small refrigerant pack Aqui-Pak 6 tube absorbent pouch UN3373 Biological Substance Category B label List of contents card FedEx return airbill and pouch FedEx Clinic Pak

Lavender Top-EDTA Tube Redraw/Take Home Kit

Quantity	Ambient Shipping Supply Components
1	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
1	Pre-printed label for blood collection tube
2	Label for handwritten Site and PIDN
2	Pre-printed labels with kit number
1	Shipping Supplies for ambient shipment of EDTA: Plastic biohazard bag with absorbent sheet Small IATA shipping box with insulated cooler Small refrigerant pack Aqui-Pak 6 tube absorbent pouch UN3373 Biological Substance Category B label List of contents card FedEx return airbill and pouch FedEx Clinic Pak

PSDC Supplemental Supply Kit

One of these will be sent to all PSDC sites with the initial shipment of kit materials.

Quantity	PSDC Supplemental Kit Components
15	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
10	Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)
5	Serum Determination (Red-Top) Blood Collection Tube (10 ml)
15	PAXgene™ Blood Collection Tube (2.5 ml)
25	Cryovial tube (2 ml) with lavender cap
10	Cryovial tube (2 ml) with red cap
10	Cryovial tube (2 ml) with blue cap
10	Cryovial tube (2 ml) with clear cap
25	Cryovial tube (2 ml) with orange cap
10	Labels for handwritten Site and PIDN
5	Cryovial tube box (holds up to 25 cryovials)
10	Individually Packaged Sterile 50ml Conical Tube
10	Plastic biohazard bag with 250 ml absorbent sheet
5	3 ½" × 24G Sprotte needle with Introducer (90mm)
5	Warning label packet
5	Bubble wrap pouch

Individual Supplies

Available upon request on the kit web-site

Quantities	Item
5, 10	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
5, 10	Sodium Heparin (Green-Top) Blood Collection Tube (10 ml)
5, 10	Serum Determination (Red-Top) Blood Collection Tube (10 ml)
5, 10	PAXgene™ Blood Collection Tube (2.5 ml)
10, 25	Cryovial tube (2 ml) with lavender cap
10, 25	Cryovial tube (2 ml) with red cap
5, 10	Cryovial tube (2 ml) with blue cap
10, 25	Cryovial tube (2 ml) with clear cap
10, 25	Cryovial tube (2 ml) with orange cap
10, 20	Labels for handwritten Site and PIDN
5, 10	Cryovial tube box (holds up to 25 cryovials)
5, 10	Individually Packaged Sterile 50ml Conical Tube
5, 10	Plastic biohazard bag with 250 ml absorbent sheet
5, 10	3 ½" × 24G Sprotte needle with Introducer (90mm)
5, 10	3 ½" x 22G Sprotte needle with Introducer (90mm)
5, 10	Warning label packet
10, 20	Bubble wrap pouch

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 so you are prepared for study visits. Please go to: http://kits.iu.edu/psdc/ to request additional kits and follow the prompts to request the desired supplies. Options include ordering specific number of kits (PSDC Blood Kit, Optional NCRAD CSF, Frozen Shipping Supply Kit, Green Top-Sodium Heparin Tube Redraw/Take Home Kit, Lavender Top-EDTA Tube Redraw/Take Home Kit and/or a PSDC Supplemental Kit) or individual 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. Please note that the centrifuge may take 30 minutes to cool, so please plan accordingly. Draw blood in the following order:

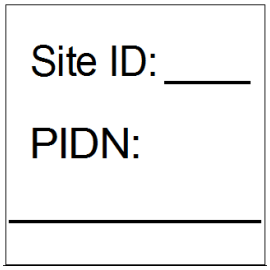
1. **EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Buffy Coat and Plasma (x3)**
2. **Sodium Heparin (Green-Top) Blood Collection Tube (10 ml) for PBMC (x2)**
3. **Serum Determination Tube (Red Top) for Serum (x1)**
4. **PAXgene™ Blood Collection Tubes for RNA (x3)**

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. Site and PIDN Label
3. Collection and Aliquot Tube Label



The **Kit Number Labels** tie together specimens collected from one subject at one visit. They are affixed on the Biological Sample and Shipment Notification Forms and on specific packing materials. Blood derivatives and CSF collected at the same visit will have **different** Kit Number Labels.

The **Site and PIDN Labels** are used to document the individual's unique PIDN. They are placed on all collection tubes.

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

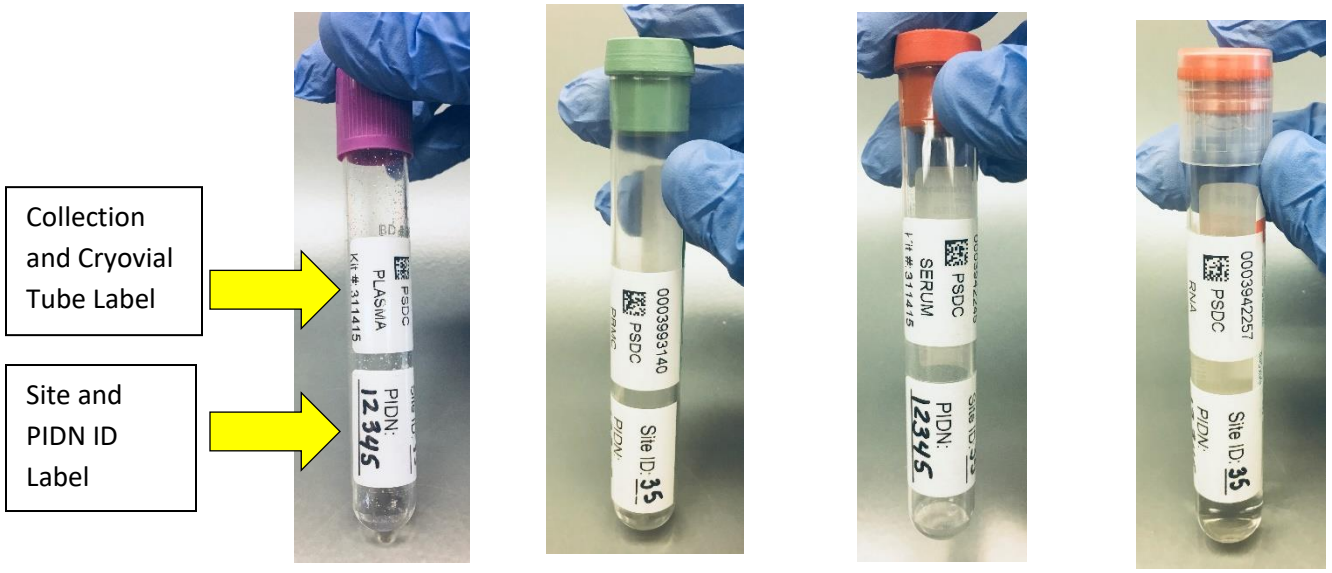
Each kit is supplied with labels for the specimens to be shipped to NCRAD. Place one Kit Number Label within the designated location on the "Biological Sample and Shipment Notification Form" and the "CSF Sample and Shipment Notification Form", if collected. Place the other Kit Number Labels on the cardboard cryobox and biohazard bag for frozen shipments, and on the lid of the shipping canisters for the Sodium Heparin tubes. See Section 8.0 for further instructions.

Place one Site and PIDN Label on each collection tube (EDTA, Sodium Heparin, Serum, and PAXgene™). Do not send blue cap conical tubes used to collect CSF to NCRAD. Discard according to Institutional guidelines. Collection and processing site staff may write on this conical tube for their own reference.

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

The Collection and Aliquot Tube Labels intended for the CSF tubes will contain a unique kit number differing from the patient's blood visit specimens.

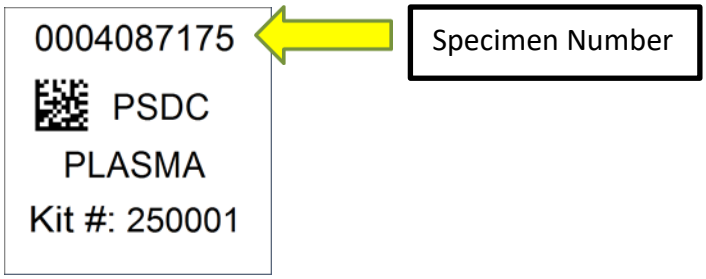
Important Note
Each collection tube will contain two labels: the Collection and Aliquot Tube Label and the Site and PIDN ID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten Site and PIDN ID label near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



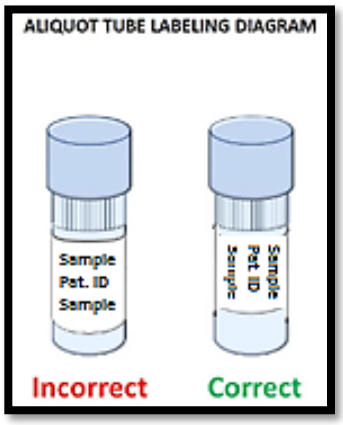
EDTA Tube | Sodium Heparin Tube | Serum Determination Tube | PAXgene™ Tube

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

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



- Using a fine point sharpie, fill-in and place the PIDN labels on the collection tubes only (EDTA, Sodium Heparin, Serum, and PAXgene™) **BEFORE** sample collection, sample processing or freezing. These labels are in addition to the Kit Number Labels. **DO NOT** place PIDN labels on any cryovials.
- The blood Collection and Aliquot Tube Labels contain a 2D barcode on the left hand side of the label. Place this barcode toward the tube cap.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see 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.

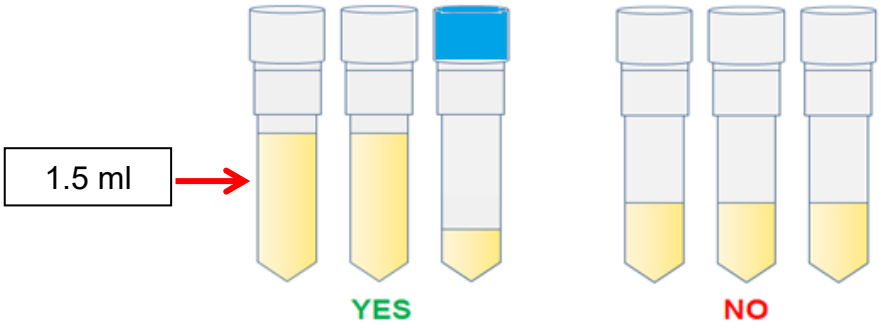


6.2 Video List

- 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_psdc.html.
  - Frozen Shipping
  - Ambient Shipping
  - Plasma and Buffy Coat Processing and Aliquoting
  - Serum Processing and Aliquoting
  - RNA (PAXgene™ Tube) Processing
  - CSF Processing and Aliquoting
  - PSDC MOP Training

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

In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to the assigned volume after processing is completed (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of sample. Aliquot the remaining biologic material as the residual volume and ship to NCRAD. Ship *all* material to NCRAD. Fill as many aliquot tubes as possible. For example, if 4.8 ml of a plasma sample is obtained, fill 3 cryovial tubes each with 1.5 ml, and one additional cryovial tube with the remaining 0.3 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 1.5 ml). Please highlight that the aliquot contains a small volume by utilizing the blue cryovial cap provided in each kit. Please record the specimen number of the residual aliquot on the Biological Sample and Notification Form.

If there are any unused cryovials, please do not send the empty cryovials to NCRAD. These unused cryovials (ensure labels are removed) can be saved as part

of a supplemental supply at your site or the cryovials can be disposed of per your site's requirements.

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

Cap Color	Sample Type
Lavender	Plasma
Clear	Buffy Coat
Red	Serum
Orange	CSF
Yellow	CSF Aliquot to local lab
Blue	Residual Aliquot (Plasma, Serum, or CSF)

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

Whole Blood Collection for Isolation of Plasma and Buffy Coat: three EDTA (Lavender-Top) Blood Collection Tubes (10 ml) (for processing of plasma aliquots and buffy coat aliquots). Three lavender-top tubes are collected at the study visit obtaining biospecimens.

1. Place completed Site and PIDN and pre-printed "**PLASMA**" collection tube label on the three EDTA (Lavender-Top) Blood Collection Tubes (10 ml). Place pre-printed "**PLASMA**" aliquot labels on the (10) 2 ml cryovial tubes with lavender caps. Place pre-printed "**BUFFY COAT**" aliquot label on the (3) 2 ml cryovials with a clear cap.

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

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

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

The following techniques shall be used to prevent possible backflow:

   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.

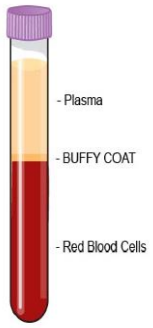
c. Release tourniquet as soon as blood starts to flow into tube.
d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

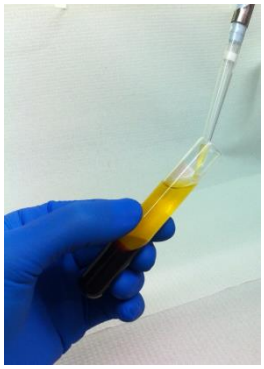
5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into each tube.

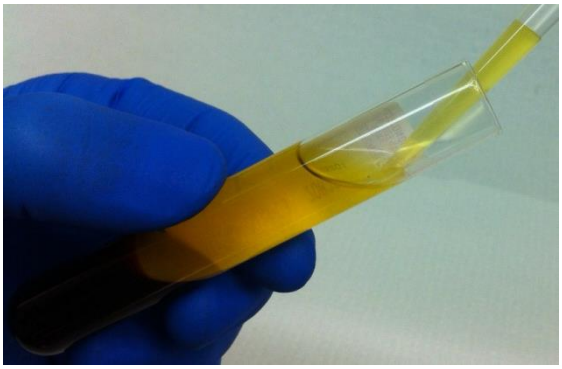
6. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8 – 10 times.**

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

8. Remove the plasma, being careful not to agitate the packed blood cells at the bottom of the collection tube, by tilting the tube and placing the pipette tip along the lower side of the wall without touching the pellet so that plasma is not contaminated by pellet material (see below). Using a graduated transfer pipette, pool plasma from all 3 EDTA tubes into the 15ml conical tube. Then, transfer plasma into the pre-labeled cryovials. Aliquot 1.5 ml per cryovial (total vials = 8-10 with 1.5 ml each). Each EDTA tube should yield, on average, 5 ml of blood plasma per tube for a total of 15 ml. Be sure to only place **plasma** in cryovials labeled with **"PLASMA"** labels**.** Take caution not to disturb the blood cells (cell pellet) at the bottom of the tube. If there is extra plasma left, use 1 extra cryovial provided for another 1.5ml aliquot of plasma. **If a residual aliquot (<1.5 ml) is created, utilize the cryovial with the blue cap to highlight which aliquot contains a smaller volume. Document the sample number on the Biological Sample and Shipment Notification Form.**

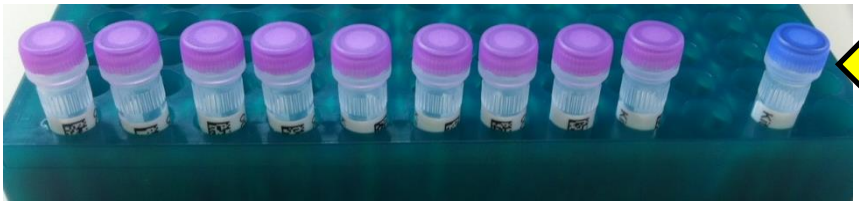






NOTE: When pipetting plasma from the plasma tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.

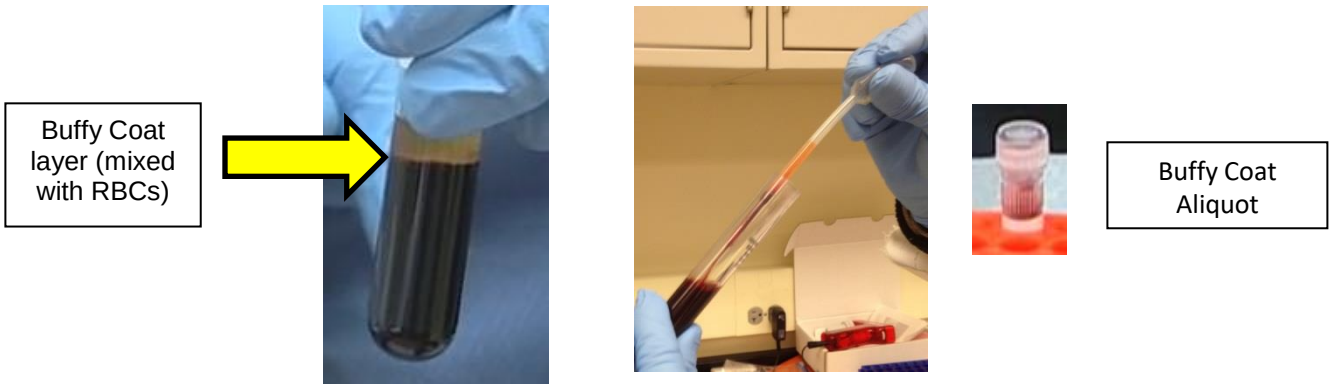
Plasma Aliquots (8-10 total possible)



Residual Aliquot with Blue Cap

9. Place the labeled cryovials in one 25-slot cryovial boxes and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C for at least 8 hours until shipped** to NCRAD on dry ice.

10. After plasma has been removed from each EDTA (Lavender-Top) Blood Collection Tube (10 ml), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) into **three** labeled cryovials with clear cap using a clean graduated transfer pipette. Buffy coat from each EDTA tube will be placed in a separate 2.0 ml cryovial with clear cap, so a total of three buffy coat aliquots will be submitted to NCRAD per participant, per visit. The buffy coat aliquot is expected to have a reddish color from the included RBCs. Be sure to place the buffy coat into the cryovial with the clear cap and "BUFFY COAT" label. Please place the buffy coat from only one blood tube in each cryovial.

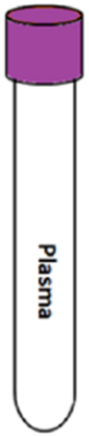


11. Dispose of collection tube with blood cell (pellet) according to your site's guidelines for disposing of biomedical waste.

12. Place the labeled cryovials in the 25-slot cryovial box with the plasma aliquots and place upright on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C for at least 8 hours until shipped** to NCRAD on dry ice.

Plasma and Buffy Coat Preparation (10ml Purple Top Tube x 3)

Step One

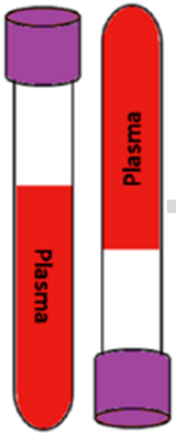


- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two

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

Step Three

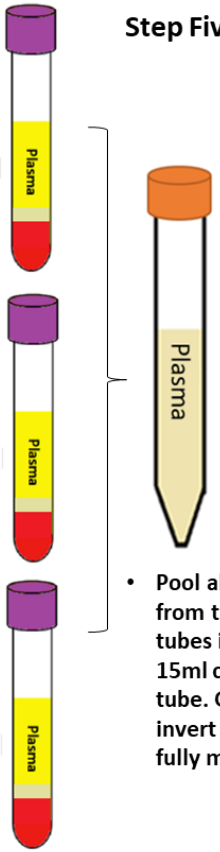


- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

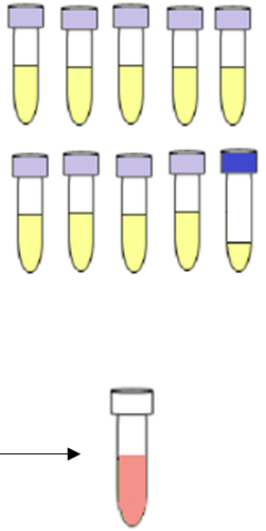
- Centrifuge samples at 1500 x g for 15 minutes at 4°C.
- EDTA tubes need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.

Step Five



- Pool all plasma from the 3 EDTA tubes into a 15ml conical tube. Gently invert 3 times to fully mix.

Step Six



- Label cryovial tubes with preprinted labels.
- Aliquot 1.5 ml into each cryovial tube.
- If residual aliquot is created, use blue cap to indicate volume difference and document Specimen Number on Biological Sample and Shipment Notification Form.
- Store plasma aliquots at -80°C until shipment.

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

6.5 Sodium Heparin (Green-Top) Blood Collection Tubes (10 ml) for PBMC

Whole Blood Collection for extraction of PBMC: Sodium Heparin (Green-Top) Blood Collection Tube (10 ml). Two green top tubes are collected at the study visit obtaining biospecimens.

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

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

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

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

The following techniques shall be used to prevent possible backflow:

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

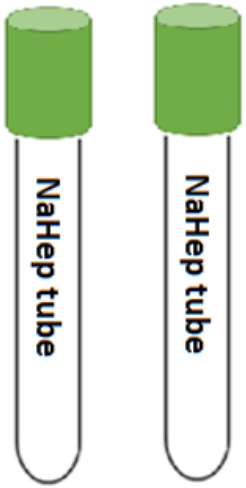
3. Allow at least 10 seconds for a complete blood draw to take place in the tube. **Ensure that the blood has stopped flowing into each tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.

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

5. Ship both unprocessed Sodium Heparin (Green-Top) Blood Collection tubes *ambient* to NCRAD the day of the participant visit. Please see Section 8.2 for detailed ambient shipping instructions.

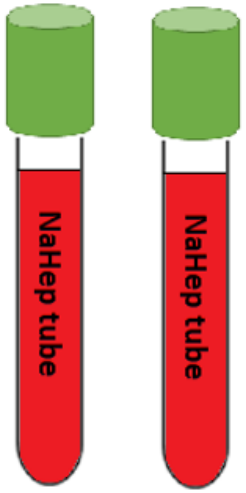
PBMC Preparation (10ml Sodium Heparin Tube x 2)

Step One



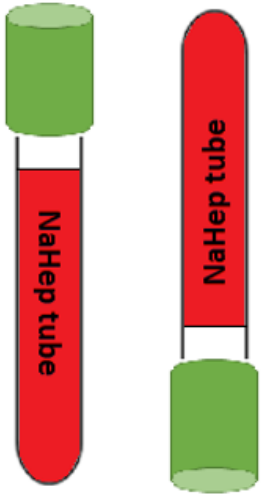
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



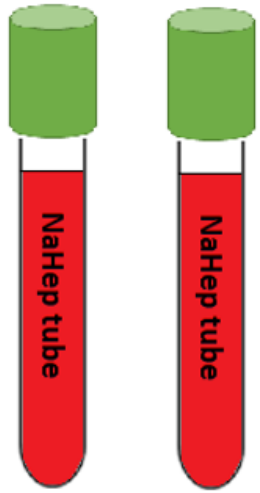
- Collect blood in Sodium Heparin Tubes allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four



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

6.6 Serum Determination (Red-Top) Tube (10 ml) for Serum

Whole Blood Collection for Isolation of Serum: Serum Determination (Red-Top) Tube (10 ml) (for processing of serum aliquots). One Red-Top tube is collected at the study visit.

1. Place completed Site and PIDN and pre-printed "SERUM" collection tube label on the red-top serum tube. Place pre-printed "**SERUM**" labels on the (3) 2 ml cryovial tubes with red caps.

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

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

4. Using a blood collection set and a holder, collect blood into**: Serum Determination (Red-Top) Tube (10 ml)** using your institution's recommended procedure for standard venipuncture technique

The following techniques shall be used to prevent possible backflow:

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

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

6. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) each tube 5 times.**

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

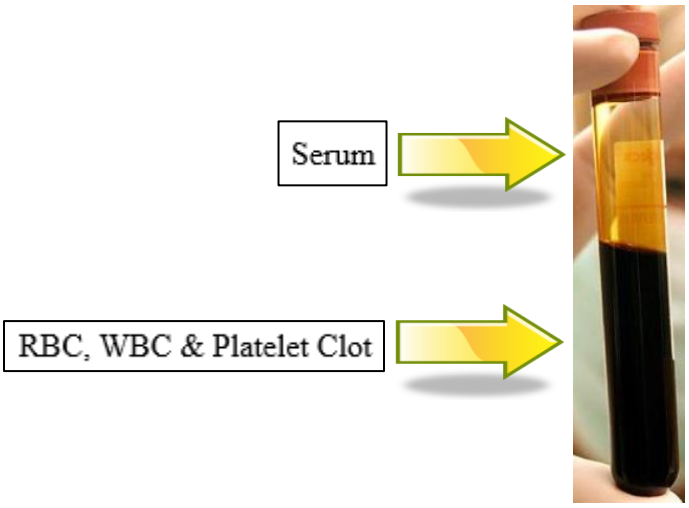
8. After 30 minutes of clotting, centrifuge the collection tube for 15 minutes at 1500 rcf (x g) at 4°C. Serum samples need to be spun, aliquoted, and stored within a -80°C within 2 hours of the time of collection. **It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum**

separation (see worksheet in Appendix A to calculate RPM with a particular rotor, or refer to: http://www.sciencegateway.org/tools/rotor.htm).

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

9. Remove the serum, being careful not to disturb the clot at the bottom of the collection tube by tilting the tube and placing the graduated transfer pipette tip along the lower side of the wall without touching the clotted pellet so that serum is not contaminated by pellet material. Using a graduated transfer pipette, transfer serum into the pre-labeled cryovials. Aliquot 1.5 ml per cryovial (total vials = 2-3 with 1.5 ml each). The red-top tube should yield, on average, 5 ml of blood serum for a total of 2-3 2 ml aliquot cryovial tubes per subject with 1.5 ml per cryovial tube. Be sure to only place **serum** in cryovials with red caps labeled with the **"SERUM"** label. If there is extra serum left, use 1 extra cryovial provided for another <1.5 ml aliquot of serum and label as appropriate. **If a residual aliquot (<1.5 ml) is created, utilize the cryovial with the blue cap to highlight which aliquot contains a smaller volume. Document the sample number on the Biological Sample and Shipment Notification Form.**

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



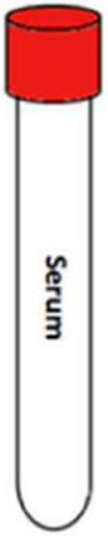
Serum Aliquots (2-4 total possible)

10. Place the labeled cryovials in the 81-slot cryobox and place upright on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C for at least 8 hours until shipped** to NCRAD on dry ice.

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

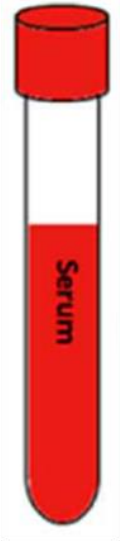
Serum Preparation (10ml Red Top Tube)

Step One



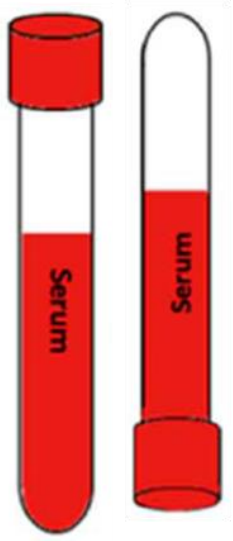
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two



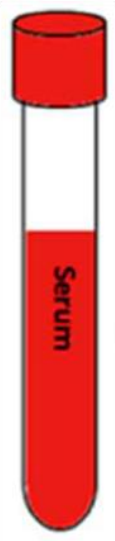
- Collect blood in Serum Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



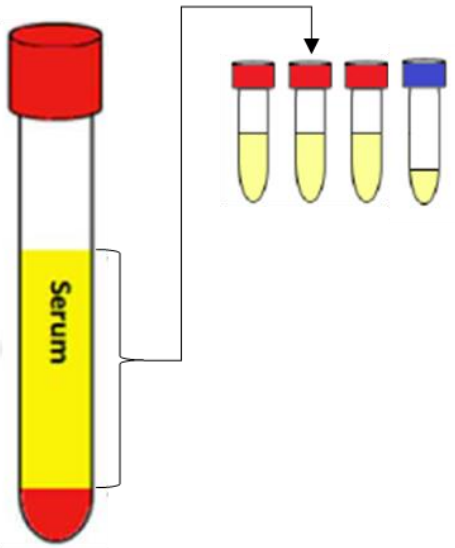
- Immediately after blood draw, invert tubes 5 times to mix samples.

Step Four



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

Step Five



- Label cryovial tubes with preprinted labels.
- Aliquot 1.5 ml into each cryovial tube.
- If residual aliquot is created, use blue cap to indicate volume difference and document Specimen Number on Biological Sample and Shipment Notification Form.
- Store serum aliquots at -80°C until shipment.

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

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

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

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

Draw the PAXgene™ tubes LAST, after all other specimens are collected for the PSDC study. The Serum Determination Tube must be the tube drawn immediately BEFORE the PAXgene™ tubes. The Serum Determination Tube draw will ensure that additives within the other collection tubes are not mixed with the PAXgene™ specimen draw.

1. **CRITICAL STEP: Store PAXgene™ Blood Collection Tubes at room temperature 64°F - 77°F (18°C to 25°C) before use.**

2. Place completed Site and PIDN label and "**RNA**" collection tube label on the PAXgene™ Blood Collection Tubes (2.5 ml) prior to blood draw; no processing is required for these tubes**; the three tubes are to be shipped to NCRAD frozen without processing at the collection site.**

3. Using a blood collection set and a holder, collect blood into the **three PAXgene™ Blood Collection Tubes** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:

   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
   e. PAXgene™ tubes should be collected LAST. Please refer to Section 6.0 for the order of the blood draw.

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

drawn into PAXgene™ blood tube(s) within the Biological Sample and Shipment Notification Form.

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

5. Place the PAXgene™ tubes upright in a **WIRE** or **PLASTIC** rack. Transfer to **-80°C Freezer when possible**. Record vial location and freezer on batch record. Store all samples at **80°C for at least 8 hours until shipped** to NCRAD on dry ice. Do **NOT** use a Styrofoam rack. This will cause the PAXgene™ tubes to crack.

RNA Preparation (2.5ml PAXgene™ Tube x 3)

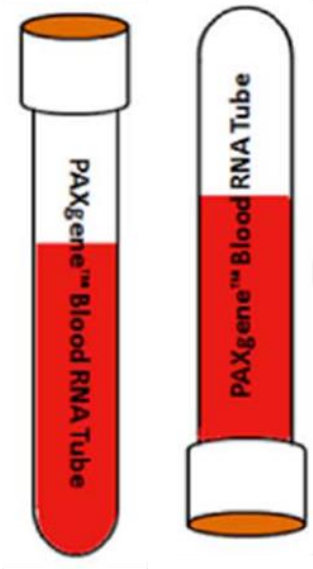
Step One

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

Step Two

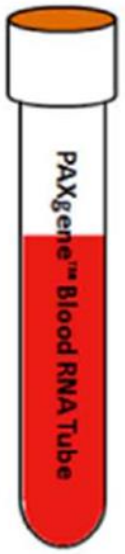
- Collect blood in PAXgene™ tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



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

Step Four



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

6.8 Sample Redraws

There may be situations that arise that require a patient sample to be redrawn from their visits. At those times, NCRAD study staff will alert site coordinators that a participant sample has failed and should be redrawn. This can happen for several reasons, including insufficient blood at the time the sample was drawn, temperature storage extremes, or even shipping errors.

Redraw kits may vary depending upon the sample that failed and must be redrawn. Tubes that may be redrawn using the redraw kit include the EDTA (Lavender-Top) Blood Collection Tube (10 ml) and the Sodium Heparin (Green-Top) Blood Collection Tube (10 ml). Both of these tubes should be sent back to NCRAD ambient and unprocessed.

Please note: The Sodium Heparin (Green-Top) Blood Collection Tubes (10 ml) (for PBMCs) in the redraw kit should not be collected on a Friday. Only draw blood for these tubes on Monday-Thursday. Always keep in mind holiday closures. Please see: https://ncrad.org/friday_blood_draws.html for a complete list of sample types and how to handle Friday Blood Draws.

Please note: The EDTA (Lavender-Top) Blood Collection Tube (10 ml) may be drawn any day of the week. If an EDTA tube is drawn on a Friday, please hold at room temperature until it can be shipped the following Monday. Samples drawn Monday-Thursday can be shipped on the same day as the blood draw.

A sample redraw may occur in one of two ways:

1. The subject travels back to the PSDC site and the coordinator redraws the blood and ships it ambient back to NCRAD.

OR

2. The site staff sends a blood kit directly to the participant's home for the blood draw to be completed by their local phlebotomist or physician. The kit is then shipped ambient by the participant or physician directly to NCRAD.

Please see Appendix E and Appendix F for Biological Shipping Forms for participants who are provided blood kits for their local physicians.

7.0 CEREBROSPINAL FLUID COLLECTION

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

CSF should be collected in the morning between 8am – 10am, preferably fasted. If fasting is not feasible, the low fat diet should be followed (See Appendix D). Record the time of last meal.

7.1 Lumbar Puncture Supplies

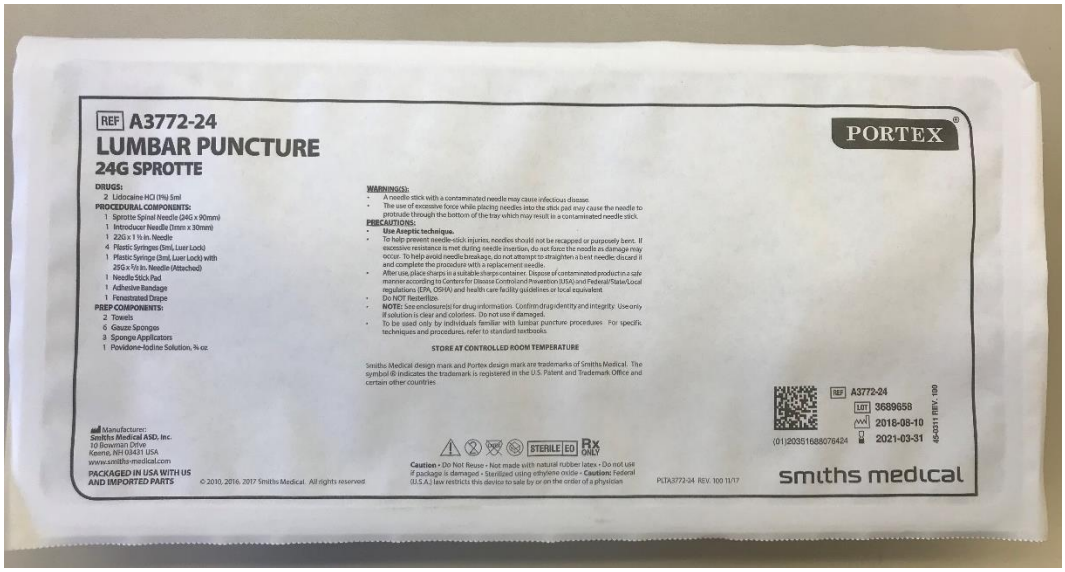
The lumbar puncture tray contains the following items, which will be used to perform lumbar puncture. Check the dates of expiration: these reflect the expiration date of the lidocaine. Supplies for collection and shipment of CSF are sent to sites in a separate kit from Indiana University.

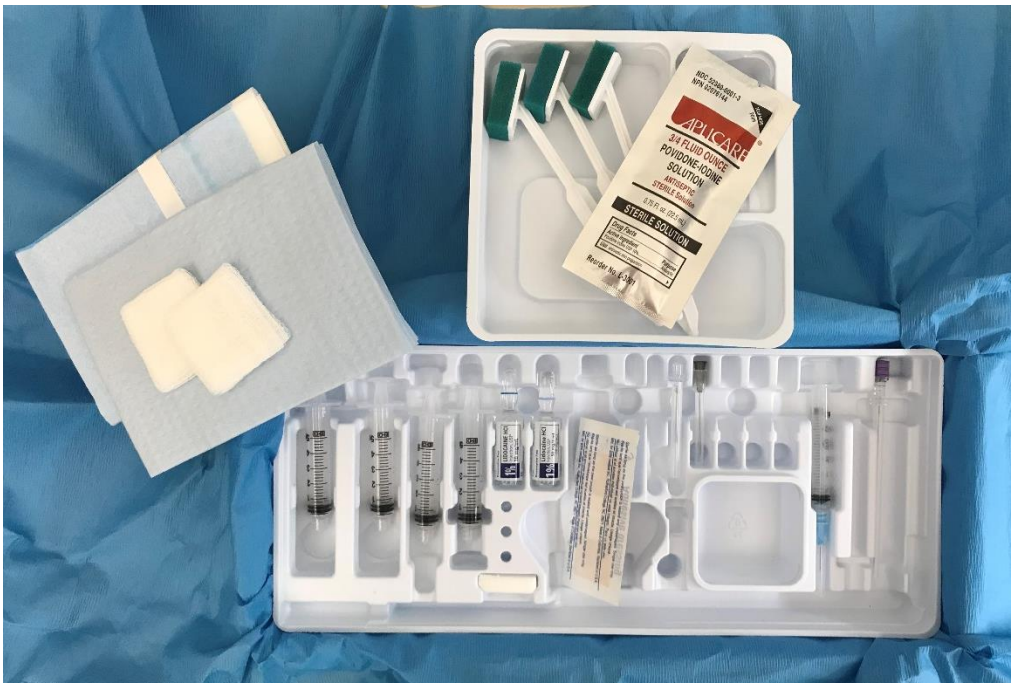
7.1.1 Lumbar Puncture Tray Components

Quantity	Lumbar Puncture Tray Kit Components
1	Sprotte needle, 24G x 90mm
1	Introducer needle, 1 mm x 30 mm
1	Hypodermic needle, 22G x 1.5”
1	Plastic syringe, (3 ml, luer lock) with 25G x 5/8” needle attached
4	Polypropylene syringe (5 ml, luer lock)
1	Needle stick pad
1	Adhesive bandage
1	Drape, fenestrated, 2 tabs, paper, 18” x 26”
2	Towel, 13.5” x 18”
6	Gauze pad, 2” x 2”
3	Sponge stick applicator
2	Lidocaine 1%, 5 ml
1	Povidone-Iodine Topical Solution, 0.75 oz

Sterile, individually packaged 50ml conical tubes are available to sites who are completing the Lumbar Puncture through the use of the gravitational method. Because not all sites are utilizing this method, the sterile conical tubes must be requested separately from the kit. They are located within the Individual Supply list of the kit request module (Please see Section 5.2).

24G Sprotte Lumbar Puncture Tray and Contents





7.2 Setting Up the LP

1. On an overbed table, remove the contents of the LP kit from the outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything wrapped until the person performing the LP is seated and begins examining the subject.

2. Feel the outside of the LP kit (still wrapped) to determine which end contains the spongy swabs. Turn this end toward the person performing the LP and begin unwrapping the kit.

3. Touch only the outside of the paper wrapper. When you grab an edge to unfold it, touch only the folded under portions of the outside of the wrapper. Also, don't let the outside of the wrapper touch any part of the inside. If you touch any part of the paper wrapper, or if any non-sterile object or outside of the wrapper touches any part of the inside of the wrapper, discard the kit and start over. If you are in doubt as to whether something touched the inside of the paper wrapper, throw the kit away and start over.

7.3 Maintaining the Sterile Field

1. Keep in mind that there is usually a lot of staff in the room during an LP, and a big part of assisting with the LP is keeping the field sterile—keeping people away from it, and reminding them to be careful around it. If anyone touches the inside of the paper wrapper or any part of the contents of the kit, throw away the kit away and start over. If there is any doubt as to whether someone touched the kit, throw it away and start over. Also, you are the monitor for whether the person performing the LP has broken sterility usually by touching something not sterile with a sterile gloved hand. Feel free to speak up and inform people if need be. Be assertive.

7.4 Tips for Clinicians Performing Lumbar Puncture

**Optimizing patient comfort and minimizing the risk of adverse events.*

1. Talk the patient through the procedure so that there are no surprises.

2. Use of a Sprotte 24g atraumatic spinal needle and careful technique are optimal for reducing post-LP headache risk. This Sprotte 24g atraumatic spinal needle is included in the NCRAD LP Tray; additional needles may be ordered upon request.
3. Use adequate local anesthesia. Use the 25g 1/2" needle and inject lidocaine to raise a skin wheal. Then, inject lidocaine using the pattern of a square— first

the center, and then to all 4 corners. If the subject is thin, do not insert the deep infiltration needle OR the spinal introducer all the way. Use only about 2/3 of their length (to prevent entering the subarachnoid space with anything other than the 24g pencil point spinal needle).

4. Increasing fluid intake immediately after LP is helpful.

5. Be sure to give post-LP care instructions verbally to the subject (see below).

7.5 Post-LP Care Instructions

- Advise the subject to refrain from exertion (e.g., exercise, housework, gardening, lifting, sexual activity, or any other strenuous activities) for 24 hours after the LP.
- Advise the subject to continue with increased fluid intake.

7.5.1 Mild to Moderate headache after a lumbar puncture

- Mild to Moderate headache following lumbar puncture usually resolves within 3-4 days.

- Treatment of Mild to Moderate headache
  - Limit physical activity as much as possible.
  - Oral fluids and caffeine are helpful. Drinking a can of Mountain Dew soft drink (for example) is preferable to coffee, which has some diuretic activity.
  - Tylenol should be used for symptomatic relief. If a subject cannot tolerate Tylenol, ibuprofen should be used. Avoid aspirin. If these do not relieve the headache, Tylenol with codeine or an equivalent could be considered.

7.5.2 Severe headache after a lumbar puncture

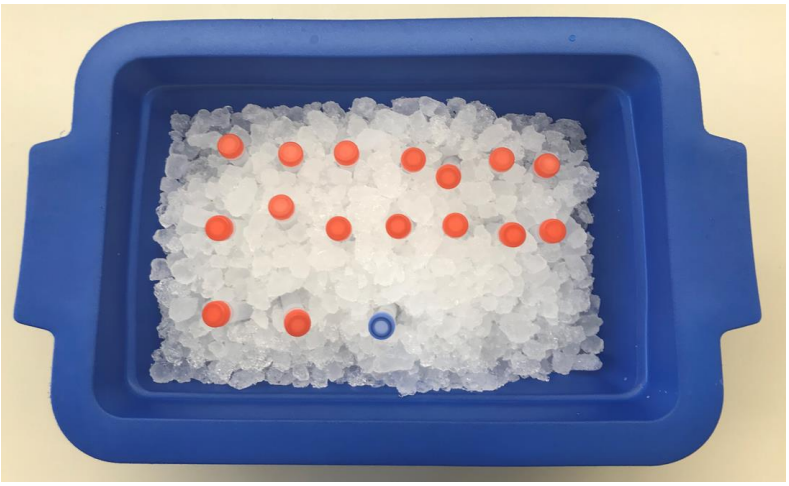
If the headache becomes severe, posturally sensitive (relieved by supine posture), or is accompanied by nausea, vomiting, tinnitus, and/or visual disturbances, the subject should contact the site study staff for further instruction per standard clinical care.

7.6 Detailed Lumbar Puncture Procedure

* See training video for *CSF Processing and Aliquoting:* http://kits.iu.edu/psdc/videos.

Place the "**CSF**" label on the collection and aliquot tubes (per Section 6.1). Prepare the 16 aliquot tubes provided by NCRAD based on the collection of ≤25 ml of CSF. Additional tubes may be necessary; these tubes may be retrieved from the PSDC Supplemental kit provided to each site.

1. Place aliquot tubes on wet ice prior to the procedure so they are pre-cooled (See below):



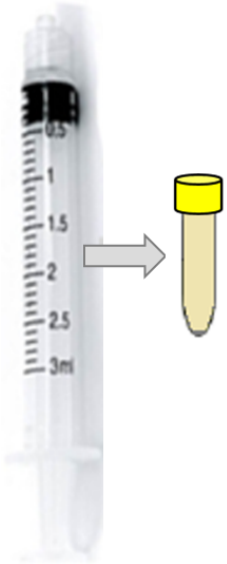
2. Perform lumbar puncture using the atraumatic technique.
3. Collect CSF into syringes or sterile conical tube (if a noticeably bloody tap, discard the first 1-2 ml). After the LP has begun and fluid is being collected, take the first 1-2 ml of CSF from the first syringe and place in the CSF labs tube (YELLOW TOP), and send it to the local lab for routine diagnostic tests.
4. Collect an additional 23 ml of CSF and transfer to 50 ml conical polypropylene tubes at room temperature. Mix gently by inverting 3-4 times. Record the time of draw (once collection is complete) on the CSF Sample and Shipment Notification Form.
5. Within 15 minutes of collection, spin the remaining CSF sample down at 2000 x g for 10 minutes at room temperature, 64°F – 77°F (18°C to 25°C). For assistance, see Appendix A.
6. Transfer the CSF with a clean pipette to a second 50ml conical tube leaving the debris at the bottom. Invert gently 3-4 times.
7. Pipette (micropipette preferred) 1.5 ml of supernatant directly into pre-cooled polypropylene CSF collection aliquot tubes (orange cap). This will yield, on average, 15-16 aliquot tubes per subject. (Use more aliquot tubes if needed; do not discard any CSF.) If there is residual amount of CSF remaining (<1.5 ml), please utilize a BLUE cap cryovial to indicate that this aliquot has low volume.
8. Within 60 minutes of CSF collection, **freeze aliquots immediately upright** on dry ice and then store at **-80°C for at least 8 hours** until ready to ship. Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the LAVA database.

CSF Preparation (20-25 mL in Syringes)

Step One

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

Step Two

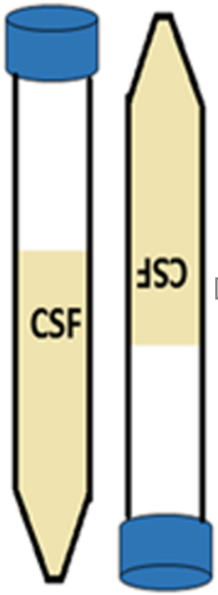


- Collect CSF into the 3mL luer lock syringe.
- Dispense 1-2mL in YELLOW cap cryovial.
- Send to local lab for testing.

Step Three

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

Step Four

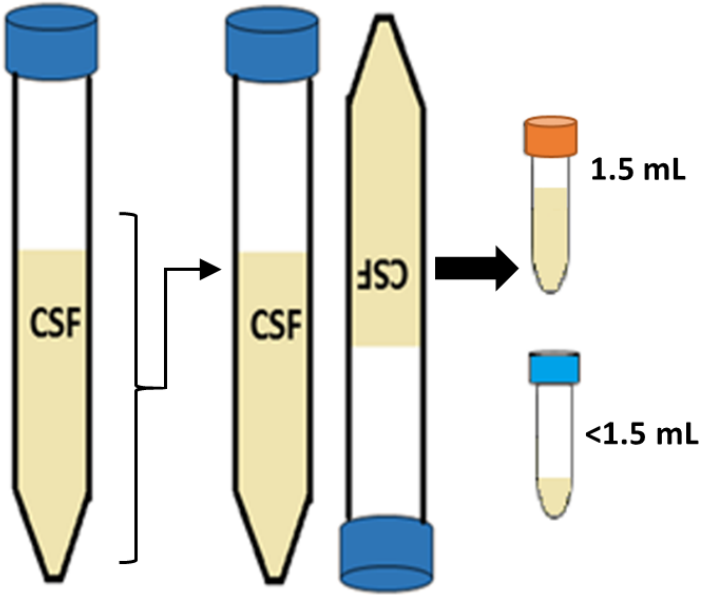


- Immediately after collection, transfer to 50 mL conical tube.
- Invert tube 3-4 times to mix sample.

Step Five

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

Step Six



- Using a clean transfer pipette, transfer all CSF into a second 50mL conical tube leaving the pellet in the bottom. Mix the second tube gently by inverting 3-4 times
- Aliquot 1.5 ml into the orange cap cryovials
- Document specimen number of residual aliquot on sample form
- Store CSF aliquots at -80° until shipment
- 1-3 mL may stay at local lab for researcher, if 20 mL of CSF is submitted to NCRAD

8.0 PACKAGING & SHIPPING INSTRUCTIONS

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your University, training and certification is available through the CITI training site (Course titled "Shipping and Transport of Regulated Biological Materials" at https://www.citiprogram.org/)

Sample Type	Processing/ Aliquoting	Tubes to NCRAD	Ship
Whole blood (Lavender-Top EDTA) for isolation of plasma & buffy coat (for DNA extraction)	1.5 ml plasma aliquots per 2 ml cryovials	8-10	Frozen
	1 ml buffy coat aliquot per 2 ml cryovial	3	Frozen
Whole blood (Green-Top Sodium Heparin) for isolation of PBMCs	N/A	2	Ambient
Whole blood (Red-Top Serum) for isolation of serum	1.5 ml serum aliquots per 2 ml cryovials	4	Frozen
Whole blood (PAXgene™) for RNA extraction	N/A	3	Frozen
CSF	1.5 ml CSF aliquots per 2 ml cryovials	16	Frozen

8.1 Frozen Shipping

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

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

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

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

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.

8.1.1 NCRAD Packaging and Shipment Instructions – Frozen Shipments

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

Attach the following to the email:

- Completed Biological Sample and Shipment Notification Form to the email notification.
  (See Appendix B and/or Appendix C for the NCRAD sample forms)

- If email is unavailable please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.

3. Place all frozen labeled 1.5 ml aliquots of plasma, buffy coat, and serum in the one 25-slot cryobox.

    i. Each cryobox holds up to 25 cryovials and there will be a maximum of 18 cryovials (10 plasma, 3 serum, and 3 buffy coat, possible 2 residuals) per blood draw (see next page).

4. Place all frozen labeled 1.5 ml aliquots of CSF in the one 25-slot cryobox.

    i. Each cryobox holds up to 25 cryovials and there will be a maximum of 17 cryovials (CSF) per draw (see next page).

Batch shipping should be performed every 3 months or when specimens from 4 participants accumulates, whichever is sooner.

25-Slot Cryobox Containing Biospecimens from One Blood Kit Visit

Place kit number label on cryobox

25-Slot Cryobox Containing Biospecimens from One CSF Kit Visit

Place kit number label on cryobox

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

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

6. As the cryoboxes and PAXgene™ tubes are placed in the large clear plastic biohazard bag, do NOT remove the absorbent material found in the bag. Seal according to the instructions on the bag. The kit number label(s) should be placed on each cardboard cryobox prior to inserting into the biohazard bag. A kit number label should also be placed on the outside of the biohazard bag.

Cryobox and PAXgene™ tubes placed in clear biohazard bag

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

8. Place the biohazard bags 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.

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

10. The inner Styrofoam shipping container must contain approximately 45 lbs (or 20kg) of dry ice. The dry ice should entirely fill the inner box and be placed on top of the biohazard bags to ensure the frozen state of the specimens.

Full Shipping Container with
Batched Samples and Dry Ice

11. 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.

12. 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.

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:

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

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

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

16. 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.

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

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

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

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

The labeled, processed, aliquoted, and frozen cryovials of plasma, buffy coat, serum, CSF, and frozen unprocessed PAXgene™ RNA tubes will be shipped to NCRAD as outlined above.

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

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

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

8.2 Ambient Shipping Instructions

*****Important Note*****
For ambient Sodium Heparin (Green-Top) Blood Collection Tube (2 x 10 ml) shipments, include no more than two tubes per shipping box. The ambient PBMC samples must be shipped the day of blood draw.
The labeled, unprocessed, sodium heparin PBMC 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!

Ambient Sodium Heparin (Green-Top) Blood Collection Tubes (2 x 10 ml) shipments should be considered as Category B UN3373 and as such must be tripled packaged and compliant with the IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

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

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

- The primary receptacle (sodium heparin tube) must be leak proof and must not contain more than 10 ml total.
- The secondary packaging (small biohazard bag) must be leak proof.
- Absorbent material must be placed between the primary receptacle (sodium heparin tube) and the secondary packaging (small biohazard bag). The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible Person
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373

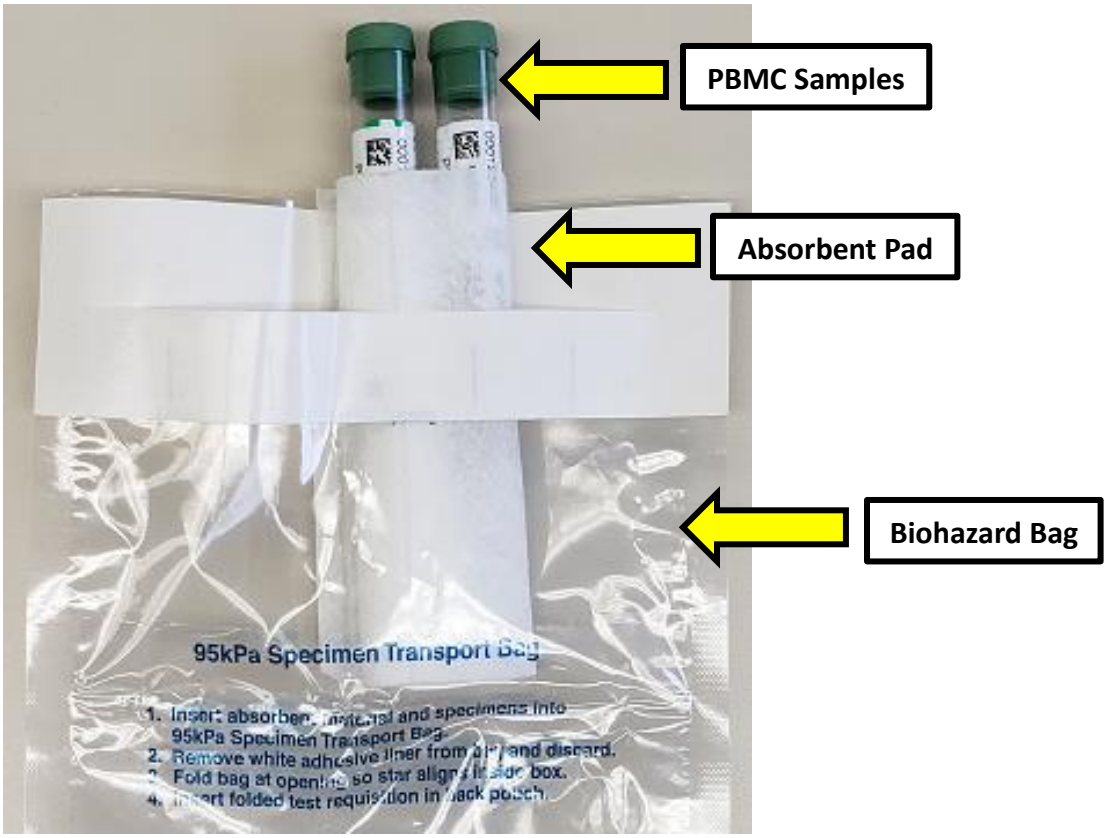
8.2.1 NCRAD Packaging and Shipment Instructions (Ambient Shipments)

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

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

3. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu
    a. Complete and attach the Biological Sample and Shipment Notification Form to the email. (See Appendix B for an example of the form)

4. Place filled and labeled sodium heparin tubes within the slots in the absorbent pad provided. The tubes and absorbent pad are then placed within a small biohazard bag with absorbent sheet.



5. Remove as much air as possible from the plastic biohazard bag, and seal the bag according to the direction printed on the bag.

6. Place Kit Number Label on outside of biohazard bag.

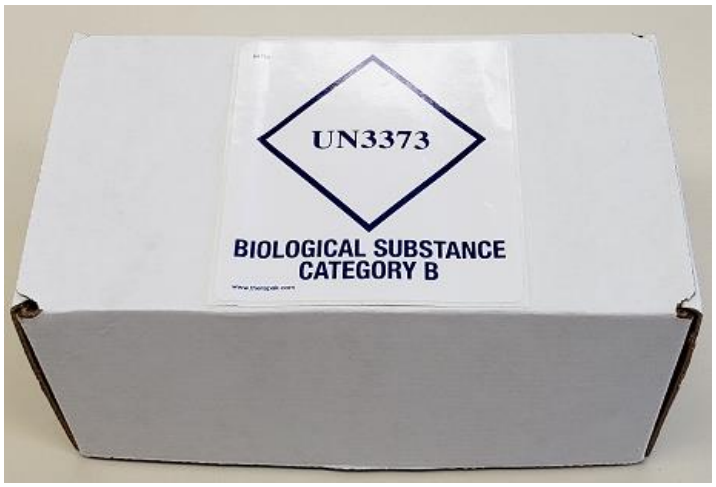
7. Place the biohazard bag in the provided small IATA Styrofoam Shipping Box.

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

9. Place the lid onto the cooler

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

11. Close shipping box. Ensure the UN3373 (Biological Substance Category B) sticker is labeled to the outside of the cardboard box and place within a Fed-Ex Clinical Pak. **Seal the Fed-Ex Clinical Pak.**



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

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

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

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

9.0 DATA QUERIES AND RECONCILIATION

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

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

10.0 APPENDICES

Appendix A
Rate of Centrifuge Worksheet

Please complete and return this form by fax or email to the NCRAD Project Manager if you have any questions regarding sample processing. The correct RPM will be sent back to you. Make note of this in your PSDC Biologics Manual.

Submitter Information

Name: **Site:**
Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

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

Calculating RPM from G-Force:

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

RCF = Relative Centrifugal Force (G-Force)
RPM = Rotational Speed (revolutions per minute)
R= Centrifugal radius in mm = distance from the center of the turning axis to the bottom of centrifuge

Comments:

Please send this form to NCRAD Study Coordinator

317-321-2003 (Fax) **alzstudy@iu.edu**

Appendix B
Biological Sample and Shipment Notification Form

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

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

General Information:

FedEx tracking #: _____

From: _____ Date: _____

Phone: _____ Email: _____

Study: PSDC PIDN: _____ Kit #: KIT BARCODE

Sex: ☐ M ☐ F Year of Birth: _____

CSF Collected? ☐ Yes ☐ No

Blood Collection:

1. Date Drawn: _____ [YYYYMMDD]
2. Time of Draw (24 hour clock): _____ [HHMM]
3. Last date subject ate: _____ [YYYYMMDD]
4. Last time subject ate (24 hour clock): _____ [HHMM]
5. Sodium Heparin (PBMC) drawn 2 x 10 ml: ☐ Yes ☐ No Original volume drawn: _____ ml
6. Total volume of blood drawn into 3 x 2.5 ml PAXgene™ RNA tubes: _____ ml
   - Were the PAXgene tubes the last tubes drawn? Yes ☐ No ☐
   - Time PAXgene™ RNA tubes placed in freezer: 24 hour clock: _____ [HHMM]

Blood Processing:

Plasma (EDTA/Lavender Top Tube)		Serum (Serum Separator/Red Top Tube)	
• 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 (3x10 ml EDTA tube):	_____ ml	Original volume drawn (1x10 ml Serum tube):	_____ ml
• Time aliquoted:	_____ [HHMM]	• Time aliquoted:	_____ [HHMM]
Number of 1.5 ml plasma aliquots created (8-10 total) **(lavender cap cryovial):**	_____ x 1.5 ml	Number of 1.5 mL serum aliquots created (2-4 total) **(red cap cryovial):**	_____ x 1.5 ml
• If applicable, volume of residual plasma aliquot (less than 1.5 ml) **(blue cap cryovial):**	_____ ml	• If applicable, volume of residual serum aliquot (less than 1.5 ml) **(blue cap cryovial):**	_____ ml
If applicable, specimen number of residual aliquot **(Last four digits):**	_____	If applicable, specimen number of residual aliquot **(Last four digits):**	_____
• 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		

Notes: _____

Appendix C

CSF Sample and Shipment Notification Form

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

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

General Information:

From: _____ Date: _____

Phone: _____ Email _____

Study: PSDC **PIDN:** _____

KIT BARCODE

Sex: ☐ M ☐ F **Year of Birth:** _____

FedEx tracking #: _____

CSF Collection:

1. Date of Collection: _____	2. Time of Collection: 24 hour clock: _____ [HHMM]
3. Last date subject ate: _____	4. Last time subject ate: 24 hour clock: _____ [HHMM]
5. Collection process: ☐ Gravity Method **OR** ☐ Aspiration	

CSF Processing:

Time spin started: 24 hour clock:	_____ [HHMM]
Duration of centrifuge:	_____ minutes
Temp of centrifuge: _____ °C	Rate of centrifuge: _____ x g
Total amount of CSF **collected** (ml):	_____ ml
Time aliquoted:	_____ [HHMM]
Number of 1.5 ml aliquots created (up to 16 total): **(Orange cap cryovials):**	_____ x 1.5 ml
If applicable, volume of CSF residual aliquot (less than 1.5 ml): **(Blue cap cryovial):**	_____ ml
If applicable, specimen number of residual aliquot tube: **(Last four digits):**	_____
Time frozen:	_____ [HHMM]
Storage temperature of freezer:	_____ °C

Notes: _____

Appendix D
Low Fat Diet Menu Suggestions

Foods to avoid prior to blood collection:

Avoid: *All Fats and nuts such as:*

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

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

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

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

All red meats or meats containing fat such as pork

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

Appendix E

Green Top-Sodium Heparin Redraw/Take Home Sample Form

TO BLOOD DRAWING PERSONNEL

This blood sample is for a study sponsored by the National Institute of Health (NIH). Samples are housed at Indiana University School of Medicine. It will need to be shipped to the address below. Please use the enclosed pre-addressed FedEx Clinical Pak.

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

The kit provided contains collection tubes with which to obtain blood from the individual for research purposes. Each kit contains 2 green-topped tubes and all necessary shipping supplies.

DO NOT REFRIGERATE; STORE AT ROOM TEMPERATURE.
DO NOT DRAW OR SHIP ON FRIDAY OR SATURDAY.
PLEASE SHIP SAME DAY AS BLOOD IS DRAWN.

Instructions for drawing and shipping blood samples:

1. Fill **GREEN TUBES** completely, if possible.
2. Invert (do not shake) tubes eight to ten times after drawing blood to thoroughly mix additive with sample.
3. **Enclose this form in shipment with sample**.
4. Ship samples by **Federal Express** immediately after drawing. Use the enclosed, pre-paid Federal Express mailer. There will be no cost to you or the patient for the shipping. Consult the enclosed information sheet for packing instructions.

KIT NUMBER (RECORDED ON LABEL): _____

PIDN (RECORDED ON LABEL): _____

STUDY SITE ID (RECORDED ON LABEL): _____

DATE BLOOD WAS DRAWN: _____

DONOR YEAR OF BIRTH: _____ DONOR SEX:_____

Appendix F

Lavender Top-EDTA Redraw/Take Home Sample Form

TO BLOOD DRAWING PERSONNEL

This blood sample is for a study sponsored by the National Institute of Health (NIH). Samples are housed at Indiana University School of Medicine. It will need to be shipped to the address below. Please use the enclosed pre-addressed FedEx Clinical Pak.

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

The kit provided contains a collection tube with which to obtain blood from the individual for research purposes. Each kit contains 1 lavender-tube and all necessary shipping supplies.

DO NOT REFRIGERATE; STORE AT ROOM TEMPERATURE.
DO NOT DRAW OR SHIP ON FRIDAY OR SATURDAY.
PLEASE SHIP SAME DAY AS BLOOD IS DRAWN.

Instructions for drawing and shipping blood samples:

1. Fill **LAVENDER TUBE** completely, if possible.
2. Invert (do not shake) tube eight to ten times after drawing blood to thoroughly mix additive with sample.
3. **Enclose this form in shipment with sample**.
4. Ship samples by **Federal Express** immediately after drawing. Use the enclosed, pre-paid Federal Express mailer. There will be no cost to you or the patient for the shipping. Consult the enclosed information sheet for packing instructions.

KIT NUMBER (RECORDED ON LABEL): _____

PIDN (RECORDED ON LABEL): _____

STUDY SITE ID (RECORDED ON LABEL): _____

DATE BLOOD WAS DRAWN: _____

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