# North American Prodromal Synucleinopathy (NAPS2)

**in collaboration with the**

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

## Biospecimen Collection, Processing, and Shipment Manual of Procedures

**Version 04.2024**

# Manual of Procedures Version 03.03.22 Summary of Changes

| Section | Change |
|---|---|
| 5.2 | Updated Visit 1-5 to "Cycle" 1-8 |
| Appendix B | Added section for Case/Control |
| Appendix C | Added section for Case/Control |

# Manual of Procedures Version 6.2.23 Summary of Changes

| Section(s) | Change |
|---|---|
| 3.0 | Updated contact information for NCRAD study coordinator |
| 5.2 | Clarified that *CSF collection highly encouraged for RBD group each cycle, but not mandatory. CSF collection is mandatory for Control group in Cycle 1 and optional in following cycles. |
| 5.2, 5.3, 6.1, 7.0, 7.5 | Removed mention of 3ml discard tube |
| 5.3 | Added *Fasting strongly recommended for blood sample collection. Participant should be fasting prior to lumbar puncture*<br><br>and<br>Added that if a blood sample is not obtained at a particular visit, this should be recorded in the notes section of the Biological Sample and Shipment Notification Form (Appendix B)**.** Submit a copy to NCRAD with a reason provided for the omission. This should also be documented in the site's NAPS2 Regulatory binder as a minor deviation. |
| 6.2 | Updated to please allow **THREE weeks** for kit orders to be processed and delivered. |
| 7.4 | Correctd that labeled cryovials should be put in the 25 slot cryobox and place on dry ice. |
| 7.5 | Added Place the PAXgene™ RNA tube upright in a **WIRE** rack at room temperature (18–25°C) for a minimum of 2 hours and a_maximum of 72 hours before processing or transferring to a −20°C freezer for 24-62 hours. Next, transfer the PAXgene™ RNA tube to a **-80°C freezer.**<br><br>**and**<br><br>Added to keep the bubble wrap sleeve from the blood kit. It will be needed to protect the PAXgene tube when sending biospecimens to NCRAD. |

| | |
|---|---|
| 8.0 | Added Anti-platelet agent (e.g. aspirin) or anticoagulant medication (e.g. warfarin (Coumadin) and/or dabigatran (Pradaxa)) is at the discretion of the local site investigator. Site specific guidelines for discontinuing asprin and anticoagulant medication for a research lumbar puncture should be followed. |
| 8.1 | Added **ASPIRATION IS A NAPS2 PROTOCOL VIOLATION** and must be reported as such. |
| 10.1 | Removed "or every three (3) months, whichever is sooner"<br>and<br>Added that if a participant has a cryobox with blood samples and a cryobox with CSF samples, this counts as two boxes towards the total of eight cryoboxes to batch ship.<br>and<br>Added that if the participant has CSF and blood samples from same cycle, both cryoboxes and PAXGene tube can be placed in the same biohazard bag and shipped to NCRAD together.<br>and<br>Deleted FedEx account #. Added to please contact NCRAD or the NAPS2 Study Team for this account number. |
| Appendix B | Updated format<br>Added date formatting under #1 and #3 in Blood Collection section<br>Added date PAXgene RNA tube placed in -80°C Freezer<br>Added time buffy coat aliquots placed in freezer<br>Added storage temperature of freezer in buffy coat section |

## Manual of Procedures Version 2.2024 Summary of Changes

| Section(s) | Change |
|---|---|
| Throughout | Updated to new NCRAD theme. |
| Throughout | Plasma aliquot sizes : 0.5 ml plasma in the first 10 aliquots and then 1.0ml aliquots for remaining plasma each into 2.0 ml orange cryovial; residual |
| Throughout | CSF aliquot sizes: 0.5 ml CSF in the first 10 aliquots and then 1.0ml aliquots for remaining CSF each into 2.0 ml orange cryovial; residual |
| Throughout | Updated "dry ice" to "pelleted dry ice" |
| Throughout | Update the name of "Site and NAPS2 ID Labels" to "NAPS2 ID Labels" since site is not not indicated on the label. |
| Throughout | Update "subject" to "participant" |
| 3.1 | Addition of alternate NCRAD phone number: 317-278-8413 |

| | |
|---|---|
| 6.1 | Blood Kits now only have 1 disposable graduated transfer pipette since sites will be expected to use a repeater pipette for plasma aliquoting. |
| 6.1 | Removed biohazard label from "Individual supplies" table |
| 7.4-7.6 | Added new step to store empty serum/EDTA/$PAXgene^{TM}$ tubes at room temperature, 64°F - 77°F (18°C – 25°C) before use. Check expiration dates on all collection tubes before visit. |
| 7.6 | Updated RNA ml PAXgene™ Tube Collection & Processing schamtic to include language to check tube expiration dates, compare the labels on the tube to the Biological Sample Form included with the kit |
| 7.6 | Removed video link because it no longer works. |
| 10.1 | Changed from step 14 to step 1: Hold packaged samples in -80C freezer until time of FedEx pick-up/drop-off. |
| 11.0 | Addition of an image to outline the amount of pelleted dry ice and the maximum number of kits each frozen shipper size should contain. |
| 11.1 | A sample shipment to NCRAD should be initiated when a study site has **four (4)** cryoboxes of samples. (updated from 5). |

# Table of Contents

# 1.0 Abbreviations

| | |
|---|---|
| **AD** | Alzheimer's Disease |
| **BL** | Baseline Visit |
| **CSF** | Cerebrospinal Fluid |
| **DNA** | Deoxyribonucleic Acid |
| **EDTA** | Ethylene Diamine Tetra-acetic Acid |
| **GUID** | Globally Unique Identifier |
| **IATA** | International Air Transport Association |
| **IUGB** | Indiana University Genetics Biobank |
| **LP** | Lumbar Puncture |
| **NCRAD** | National Centralized Repository for Alzheimer's Disease and Related Dementias |
| **PHI** | Protected Health Information |
| **RBCs** | Red Blood Cells |
| **RBD** | REM Sleep Behavior Disorder |
| **RCF** | Relative Centrifugal Force |
| **RPM** | Revolutions Per Minute |

# 2.0 Purpose

The purpose of this manual is to provide NAPS2 staff (PIs, study coordinators, and the sample collection and processing teams) at the various study sites with instructions for collection and submission of biological samples for NAPS2 study cycles. 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 cycle:

- Serum
- Plasma
- Buffy Coat (DNA Extraction)
- RNA
- CSF

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 specimens to be submitted to NCRAD for the NAPS2 protocols.

# 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

**General NCRAD Contact Information**
Phone: 1-800-526-2839
Alternate phone: 317-278-8413
Email: alzstudy@iu.edu
Website: www.ncrad.org
NAPS2 Study Specific Webpage: https://www.ncrad.org/resource/naps2.html

**Abigail Erickson, BS, CCRP, Study Coordinator**
Phone: 317-278-1133
Email: agericks@iu.edu

**Sample Shipment Mailing Address**
NCRAD
Indiana University School of Medicine
351 W. 10th Street
TK-217
Indianapolis, IN 46202

## 3.2 NCRAD 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 NCRAD 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 |
| June 19 | Juneteenth |
| July 4 | Independence Day |
| 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 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.**

- 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.**

## 4.0 GUID

The GUID is a participant ID that allows researchers to share data specific to a study participant, without exposing personally identifiable information. It is mandatory for NAPS2 that a GUID is included on the biospecimen shipping form (NAPS2 source document section N15 pg1 and N16 pg1). A GUID is made up of random alpha-numeric characters and does not include any PHI in the identifier. By using GUIDs in your research data, the system can associate a single research participant's genetic, imaging, and clinical assessment data even if the data was collected at different locations or throughout different studies.

To create a GUID follow these steps:

1. Create an account: https://bricsguid.nia.nih.gov/portal/jsp/login.jsp
2. Once you have an account, go to the GUID Tool – Create GUID
3. To open the 'Launch GUID Tool' you will need to have Java installed on your device
4. In order to generate a GUID, the following PHI is required (Appendix D):
   - Complete legal given (first) name of participant at birth
   - If the participant has a middle name
   - Complete legal family (last) name of participant at birth

- Day of birth
- Month of birth
- Year of birth
- Name of city/municipality in which participant was born
- Country of birth

# 5.0 NCRAD Laboratory Information

## 5.1 Site Required Equipment

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

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquet
- Alcohol Prep Pad
- Gauze Pad
- Bandage
- Butterfly needles and hub
- Microcentrifuge tube rack
- Sharp's bin and lid
- Wet Ice Bucket
- Wet ice
- Pelleted dry ice
- Repeater pipette or PIPETMAN

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 ≥ 2000 x g with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:

- Pelleted dry ice (about approximately 30-45 lbs per shipment)

## 5.2 mL and uL Coversion Chart

Measurements are listed in both mL and uL in this MOP.

1mL = 1,000uL

$$\_\_\_\_\_\text{mL} \times \frac{1{,}000\text{uL}}{1\text{mL}} = \_\_\_\_\_\text{uL}$$

1uL = 0.001mL

$$\_\_\_\_\_\text{uL} \times \frac{0.001\text{mL}}{1\text{uL}} = \_\_\_\_\_\text{mL}$$

## 5.3 Biospecimens Sent to NCRAD

Biospecimens collected include whole blood and CSF

| | Cycle 1 | Cycle 2 | Cycle 3 | Cycle 4 | Cycle 5 | Cycle 6 | Cycle 7 | Cycle 8 |
|---|---|---|---|---|---|---|---|---|
| **Serum** | X | X | X | X | X | X | X | X |
| **Plasma** | X | X | X | X | X | X | X | X |
| **Buffy Coat** | X | X | X | X | X | X | X | X |
| **RNA** | X | X | X | X | X | X | X | X |
| **CSF*** | X | X | X | X | X | X | X | X |

*CSF collection highly encouraged for RBD group each cycle, but not mandatory. CSF collection is mandatory for Control group in Cycle 1 and optional in following cycles.

The 10ml red-top serum tube and 10ml EDTA tubes are processed locally into serum, plasma and buffy coat fractions; they are then aliquoted, frozen at the study site, and shipped on pelleted dry ice to NCRAD. The 2.5ml PAXgene™ tube is frozen locally without further processing.

CSF will be collected and aliquoted locally, frozen at the study site, and then shipped on pelleted dry ice to NCRAD.

Frozen samples are to be submitted according to the shipping methods outlined in Section 10.1. Guidelines for the processing, storage location, and timing of sample collection are listed in the following tables.

## 5.4 Biospecimen Collection Charts

### *5.4.1 Biospecimen Collection for Cycles 1-8: RNA, Serum, Plasma and Buffy Coat Isolation*

***Fasting strongly recommended for blood sample collection. Participant should be fasting prior to lumbar puncture***

| Sample Type | Collection Tube | Processing/ Aliquoting | Tubes to NCRAD | Ship |
|---|---|---|---|---|
| **Whole blood for isolation of Serum** | 1 x Plain Red-Top Serum Blood Collection Tube (10ml) | 1.5 ml serum aliquot per 2.0 cryovial (red cap).<br><br>Rresidual volume placed in 2.0 cryovial with blue cap. | Up to 4 | Frozen |
| **Whole blood for isolation of plasma and buffy coat** | 4 x EDTA (Lavender-Top) Blood Collection Tube (10 ml) | PLASMA:<br>10 x 0.5ml aliquots in 2.0ml green cap cryovials.<br><br>15 x 1.0ml aliquots in 2.0ml purple cap cryovials.<br><br>Rresidual volume placed in 2.0 cryovial with blue cap. | Up to 26 | Frozen |
| | | BUFFY COAT:<br>Aliquot buffy coat from each (4) EDTA tube into its own 2.0ml clear cap cryovial | Up to 4* | Frozen |
| **Whole blood for RNA extraction** | 1 x PAXgene™ Blood Collection Tube (2.5 ml) | N/A | 1 | Frozen |

*Sites may elect to keep 1-2 buffy coats per participant locally

If a blood sample is not obtained at a particular visit, this should be recorded in the notes section of the **Biological Sample and Shipment Notification Form** (Appendix B)**.** Submit a copy to NCRAD with a reason provided for the omission. This should also be documented in the site's NAPS2 Regulatory binder as a minor deviation.

### *5.4.2 Biospecimen Collection for CSF*

| Sample Type | Collection Tube | Number of Tubes Supplied in Kit | Processing/ Aliquoting | Tubes to NCRAD | Ship |
|---|---|---|---|---|---|
| **CSF** | Sterile Containers<br><br>(20-30 ml CSF) | 37 | CSF:<br><br>10 x 0.5ml aliquots in 2.0ml green cap cryovials.<br><br>25 x 1.0ml aliquots in 2.0ml orange cap cryovials.<br><br>residual volume placed in 2.0ml cryovial with blue cap.<br><br>1-2ml for local lab placed in 2.0 ml cryovial with yellow cap. | Up to 36 | Frozen |

# 6.0 Specimen Collection Kits, Shipping Kits, and Supplies

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD, the Blood Supplemental Supply Kit, and the Frozen Shipment Kit; 2) 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 pelleted dry ice and equipment supplies listed in Section 5.1). These materials include blood tubes, pipettes, LP trays (when applicable), boxes for RNA/serum/plasma/buffy coat/CSF aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, NAPS2 ID Labels, Collection and Aliquot Tube Labels will all be provided by NCRAD. Details regarding the blood and CSF Kits are found in this Manual of Procedures. Collection and Aliquot Tube Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment.

## 6.1 Specimen Collection Kit Contents

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

## NAPS2 Blood Collection Kit

| Quantity | Blood Collection Kit Components |
|---|---|
| 1 | Plain Red Top Serum (Red-Top) Blood Collection Tube (10 ml) |
| 4 | EDTA Lavender Top Blood Collection Tube (10 ml) |
| 1 | PAXgene™ Blood Collection Tube (2.5 ml) |
| 1 | 50-ml conical polypropylene tube-individually wrapped |
| 10 | Cryovial (2.0 ml) with green cap |
| 15 | Cryovial (2.0 ml) with lavender cap |
| 4 | Cryovial (2.0 ml) with clear cap |
| 2 | Cryovial (2.0 ml) with blue cap |
| 3 | Cryovial (2.0 ml) with red cap |
| 1 | Disposable graduated transfer pipette |
| 40 | Pre-printed Collection and Aliquot Tube Label |
| 3 | Pre-printed Kit Number Label |
| 7 | Labels for Handwritten NAPS2 ID |
| 1 | Microcentrifuge box (81-slot) |
| 1 | Labeled Resealable bag |
| 1 | Bubble wrap tube sleeve |

## NAPS2 CSF Kit

| Quantity | CSF Kit Components |
|---|---|
| 10 | Cryovial tube (2.0 ml) with green cap |
| 25 | Cryovial tube (2.0 ml) with orange cap |
| 1 | Cryovial tube (2.0 ml) with yellow cap |
| 1 | Cryovial tube (2.0 ml) with blue cap |
| 2 | 50-ml conical polypropylene tube-individually wrapped |
| 36 | Pre-printed CSF collection and Aliquot Tube Label |
| 2 | Pre-printed Kit Number label |
| 2 | Pre-printed NAPS2 ID labels |
| 1 | Microcentrifuge box (81-slot) |
| 1 | Labeled resealable bag |

**NAPS2 LP Kit**

| Quantity | LP Kit Components |
|---|---|
| 1 | Sprotte needle, 22 or 24 gauge X 3.5" (90mm) |
| 1 | Introducer needle, 1 mm x 30 mm |
| 1 | Hypodermic needle, 22 gauge 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 |

**NAPS2 Supplemental CSF Kit**

| Quantity | CSF Supplemental Supply Kit Components |
|---|---|
| 5 | 50-ml conical polypropylene tube-individually wrapped |
| 50 | Cryovial tube (2.0 ml) with orange cap |
| 30 | Cryovial tube (2.0 ml) with green cap |
| 5 | Cryovial tube (2.0 ml) with blue cap |
| 5 | Cryovial tube (2.0 ml) with yellow cap |
| 5 | 3 ½" × 22 (or 24) Sprotte needle with Introducer (90mm) |

**NAPS2 Blood Supplemental Supply Kit**

| Quantity | Blood-Based Supplemental Supply Kit Components |
|---|---|
| 20 | EDTA (Lavender-Top) Blood Collection Tube (10 ml) |
| 5 | PAXgene™ Blood Collection Tube (2.5 ml) |
| 5 | Plain Red Top Serum (Red-Top) Blood Collection Tube (10 ml) |
| 5 | 50-ml conical polypropylene tube-individually wrapped |
| 20 | Cryovial tube (2.0 ml) with green cap |
| 25 | Cryovial tube (2.0 ml) with lavender cap |
| 10 | Cryovial tube (2.0 ml) with blue cap |
| 10 | Cryovial tube (2.0 ml) with clear cap |
| 10 | Cryovial tube (2.0 ml) with red cap |
| 20 | Disposable graduated transfer pipette |
| 20 | Labels for handwritten NAPS2 ID |
| 5 | Microcentrifuge box (81-slot) |
| 3 | Pre-printed airbills/shipping labels/plastic protective pouches |
| 3 | Warning Label Package |
| 8 | Small Biohazard bag with absorbent sheet |
| 2 | Fine Point Marker Pens |

**NAPS2 Large Frozen Shipping Kit**

| Quantity | Frozen Shipping Kit Components |
|---|---|
| 4 | Plastic Biohazard bag with absorbent sheet (large) |
| 1 | FedEx return airbill & plastic protective pouch |
| 1 | Shipping box/Styrofoam container - large |
| 1 | Warning label packet with dry ice sticker |

**NAPS2 Small Frozen Shipping Kit**

| Quantity | Frozen Shipping Kit Components |
|---|---|
| 2 | Plastic Biohazard bag with absorbent sheet (large) |
| 1 | FedEx return airbill & plastic protective pouch |
| 1 | Shipping box/Styrofoam container - small |
| 1 | Warning label packet with dry ice sticker |

**Individual Supplies**

| Quantities | Items Available upon request within the NCRAD kit module. |
|---|---|
| By Request | Microcentrifuge box (81-slot) |
| By Request | Sprotte needle, 22 or 24 gauge X 3.5" (90mm) |
| By Request | Sprotte needle, 22 or 24 gauge X 4.75" (120mm) |
| By Request | Cryovial tube (2.0 ml) with lavender cap |
| By Request | Cryovial tube (2.0 ml) with orange cap |
| By Request | Cryovial tube (2.0 ml) with yellow cap |
| By Request | Cryovial tube (2.0 ml) with blue cap |
| By Request | Cryovial tube (2.0 ml) with clear cap |
| By Request | 50-ml conical polypropylene tube-individually wrapped |
| By Request | 15-ml conical polypropylene tube-individually wrapped |
| By Request | FedEx return airbill & plastic protective pouch |
| By Request | Shipping container for dry ice shipment |
| By Request | Plastic biohazard bag with absorbent sheet (large) |
| By Request | Disposable graduated transfer pipette |
| By Request | PAXgene™ Blood Collection Tube (2.5 ml) |
| By Request | Plain Red Top Serum (Red-Top) Blood Collection Tube (10 ml) |
| By Request | EDTA (Lavender-Top) Blood Collection Tube (10 ml) |
| By Request | Warning label packet |
| By Request | UN3373 label |
| By Request | Dry ice shipping label |
| By Request | Fine Point Marker Pens |
| By Request | NAPS2 ID Labels |
| By Request | Bubble wrap tube sleeve |

## 6.2 Kit Supply to Study Sites

Each individual site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits. Please go to www.kits.iu.edu/NAPS2 to request additional kits and follow the prompts to request the desired supplies. Options include ordering a specific number of kits; we are also including the option of simply ordering the desired amount of extra supplies.

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

# 7.0 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. Plain Red Top Serum Blood Collection Tube (10 ml) for Serum for NCRAD
2. Any tubes for internal studies (optional) (this includes optional PT/INR and/or CBC only)
3. EDTA (Lavender-Top) Blood Collection Tubes (10 ml) for Plasma and Buffy Coat for NCRAD x4
4. PAXgene™ Blood Collection Tube (2.5 ml) for NCRAD

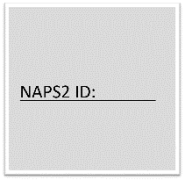
Specific instructions for collection and processing of each sample are detailed on the following pages.

## 7.1 Labeling Samples

### *7.1.1 Label Type Summary*

1. Kit Number Label
2. NAPS2 ID Label
3. Collection Tube and Aliquot Label


**Kit Number Labels** tie together all specimens collected from one participant at one visit. They should be placed on each cryobox, and in the designated location on the Blood Sample and Shipment Notification Forms.

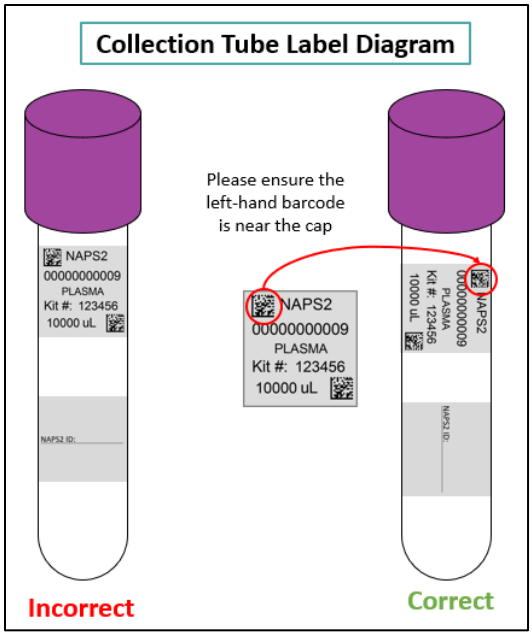



**NAPS2 ID Labels** are used to document the individual's unique NAPS2 ID. Place one label on each blood collection tube.


Place one **Collection Tube and Aliquot Label** on each blood collection tube and cryovial.

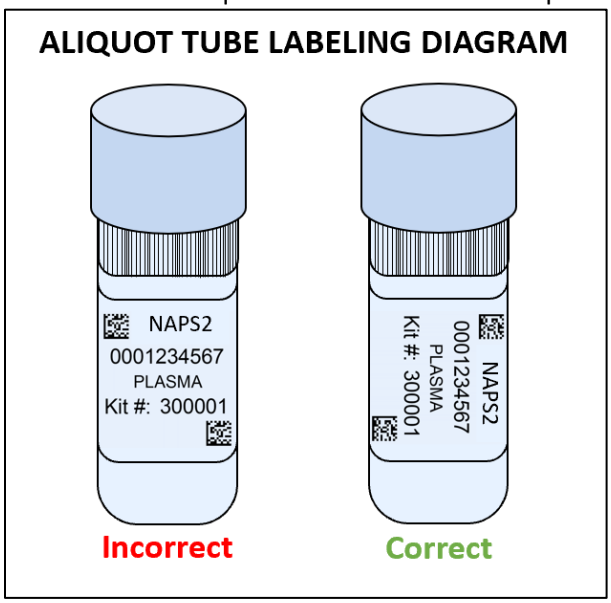
### *7.1.2 Labeling Collection Tubes*

**Each collection tube will contain two labels**: the Collection Tube Label and the NAPS2 ID Label. Be sure to place labels in the same configuration consistently among tubes, with Collection Tube Label near the top of the tube and the handwritten NAPS2 ID Label near the bottom of the tube.




### *7.1.3 Labeling Aliquot Tubes*

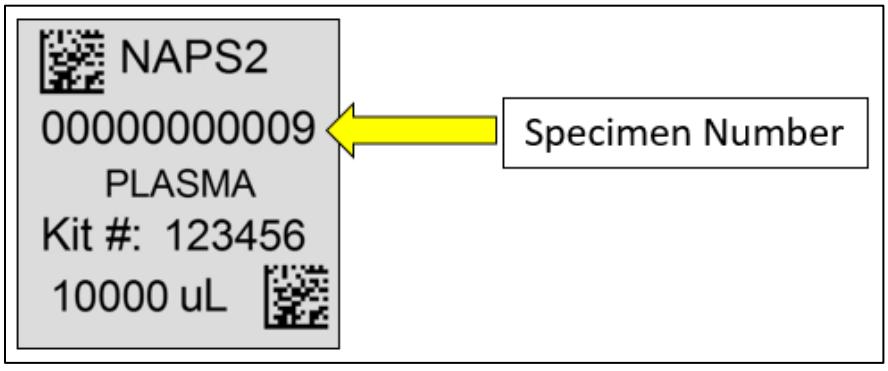
**Each <u>aliquot tube</u> will contain one label**: the Aliquot Tube. Be sure to place the label in the same configuration consistently among tubes, with the left hand barcode of the Aliquot Tube Label near the top of the tube.



Collection and Aliquot tube labels include the type of specimen on the label. The chart below summarizes the association between cap color and type of sample.

| Cap Color | Sample Type |
|---|---|
| Red Cap | Serum |
| Green Cap | 0.5 ml aliquots (plasma and CSF) |
| Lavender Cap | 1 ml Plasma aliquots |
| Clear Cap | Buffy Coat |
| Blue Cap | Residual (serum, plasma or CSF) |
| Orange Cap | 1 ml CSF aliquots |
| Yellow Cap | CSF to Local Lab |

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



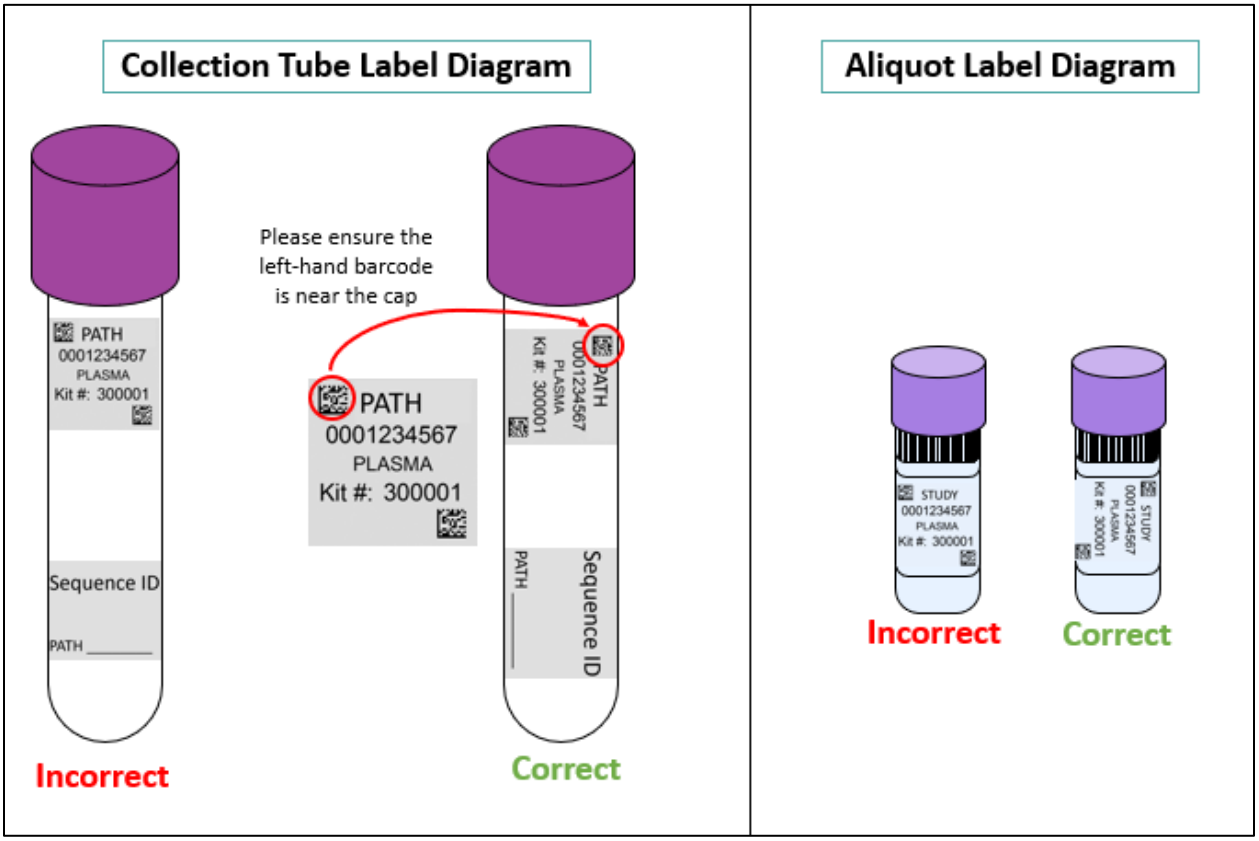

If there are any unused cryovials, please do not send the empty cryovials to NCRAD. These unused cryovials (ensure labels are removed) can be saved as part of a supplemental supply at your site or the cryovials can be disposed of per your site's requirements.

### 7.1.4 *Labeling Specimen Tubes*

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

- Place Collection Tube and Aliquot Labels on **ALL** collection tubes and cryovials **BEFORE** sample collection. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Using a fine point permanent marker, fill-in and place the NAPS2 Labels on the EDTA (purple-top) tubes **BEFORE** sample collection. These labels are placed on collection tubes in addition to the Collection Tube Label.
- The Collection Tube Labels contain a 2D barcode on the left-hand side of the label. Place this barcode toward the tube cap.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright).

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. The following pictures show the correct orientation of the labels on the collection tubes and cryovials.



## 7.2 Video List

The following training videos are available to assist you with the specimen processing, aliquoting, and shipping processes. The videos are available at: https://ncrad.iu.edu/resource/naps2.html

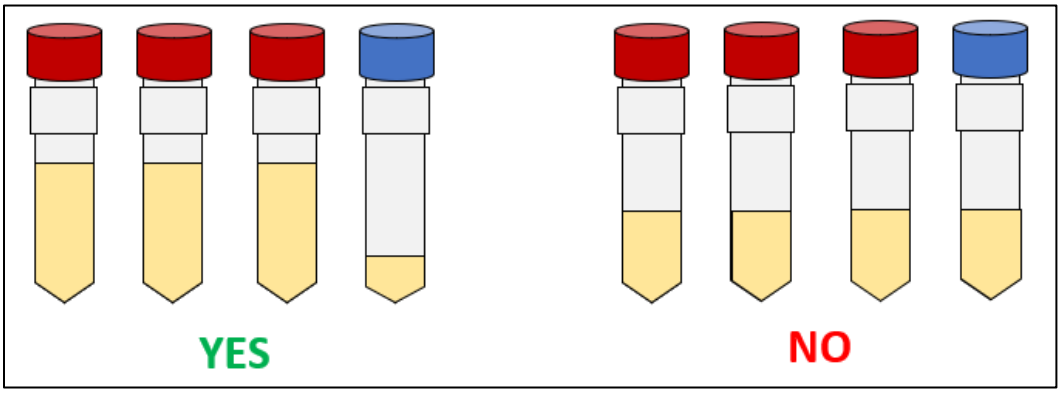
- NAPS2 MOP Training
- Serum, Plasma and Buffy Coat Processing and Aliquoting
- Frozen Shipping

## 7.3 Filling Aliquot Tubes (Plasma & 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 cryovial should be filled to the assigned volume with the respective biological material after processing is completed (refer to detailed processing instructions for average yield per sample).

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

Aliquot the remaining biologic material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 5 ml of serum is obtained, you should fill the 3 red-cap cryovials with 1.5 ml, and a blue-cap cryovial with 0.5 ml.




**Please note:** It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume. Please record the specimen number and volume of the residual aliquot on the Biological Sample and Notification Form.

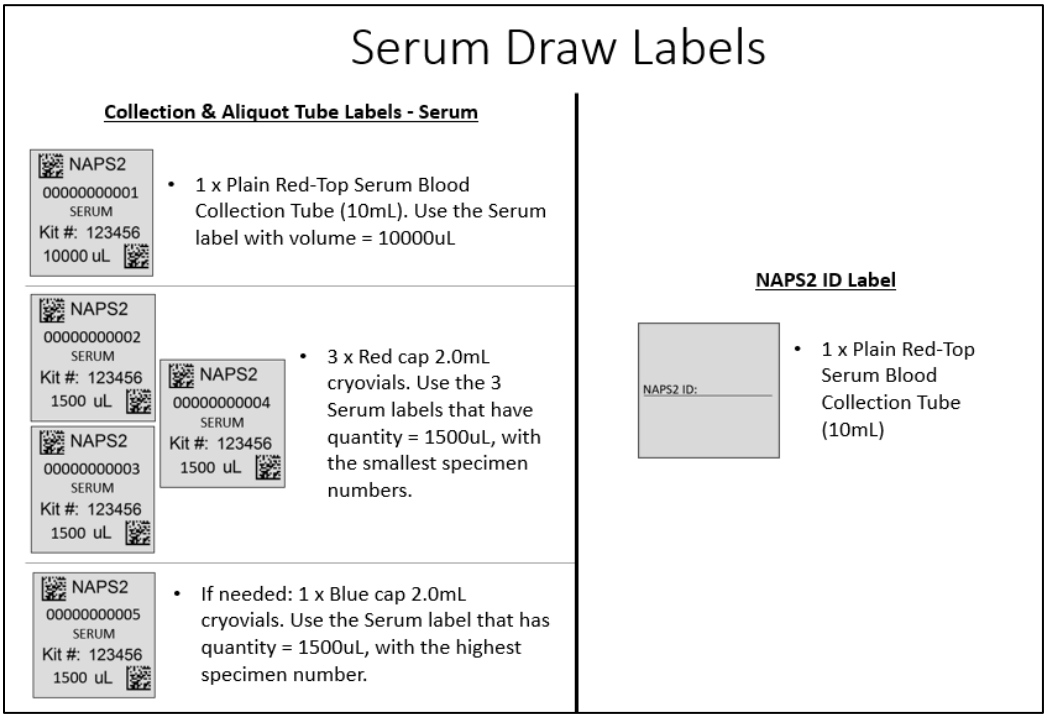
## 7.4 Plain Red-Top Serum Blood Collection Tube (10 ml) for Serum

**Whole Blood Collection for Isolation of Serum: Plain Red-Top Serum Blood Collection Tube (10 ml) (for processing of serum aliquots)**

1. Store empty serum tubes at room temperature, 64°F - 77°F (18°C – 25°C) before use. Check expiration dates on all collection tubes before visit.

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

3. Locate and verify proper labeling for Collection and Aliquot **"SERUM"** Tube Labels on the Plain Red-Top Serum Blood Collection Tube,the (3) 2.0 ml cryovial tubes with red caps, and (1) 2.0 ml cryovial with blue cap (if necessary, for residual).




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

   **The following techniques shall be used to prevent possible backflow:**
   a. Place participant's arm in a downward position.
   b. Hold tube in a vertical position, below the participant's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into last 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 each tube. **Ensure that the blood has stopped flowing into each tube before removing the**

**tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.

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

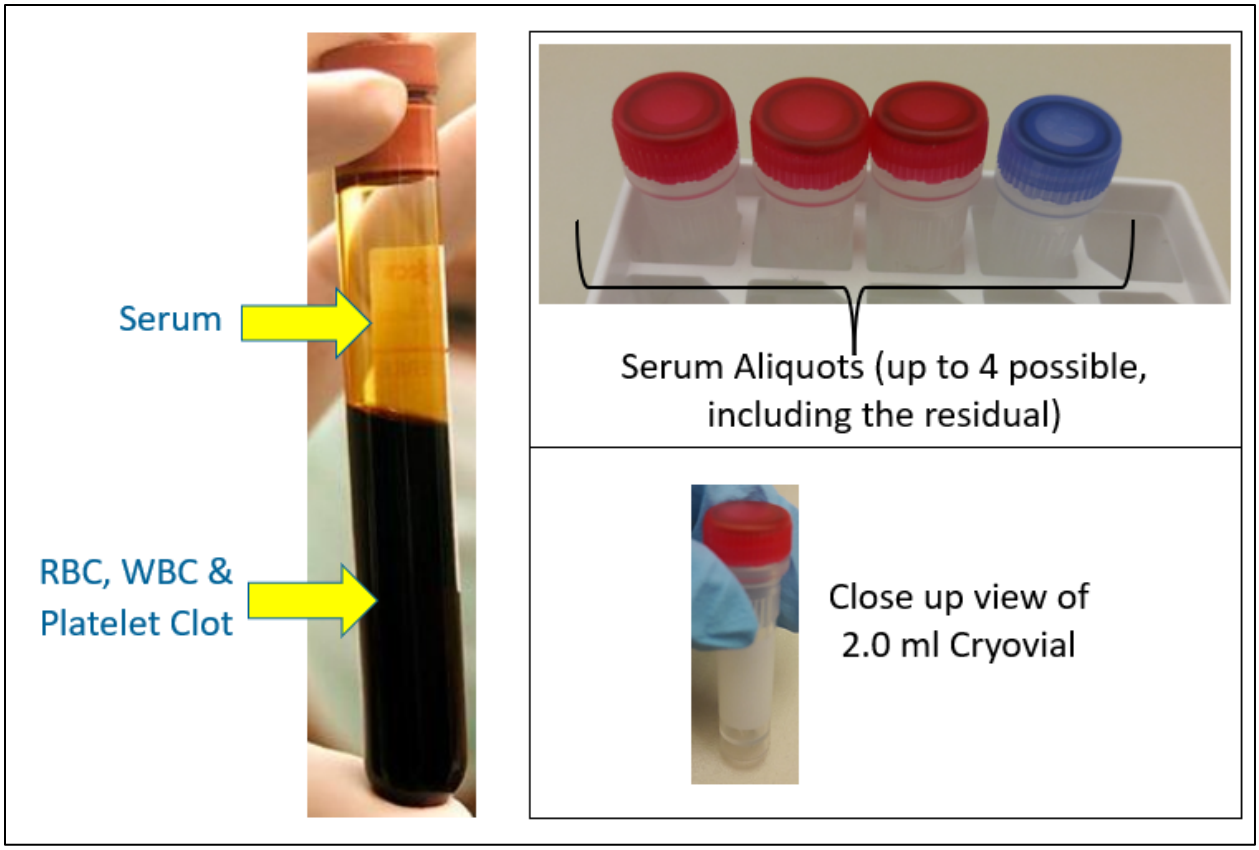
6. **CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) each tube 5 times.**

   **CRITICAL STEP: Allow blood to clot at room temperature by placing it upright in a vertical position in a tube rack for 30 minutes. If sample is not clotted allow it to set up to 60 minutes to clot. Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.**

7. After 30 minutes of clotting, centrifuge the collection tube for 10 minutes at 2000 x g at 4°C. **It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum separation (see worksheet in Appendix A to calculate RPM)**

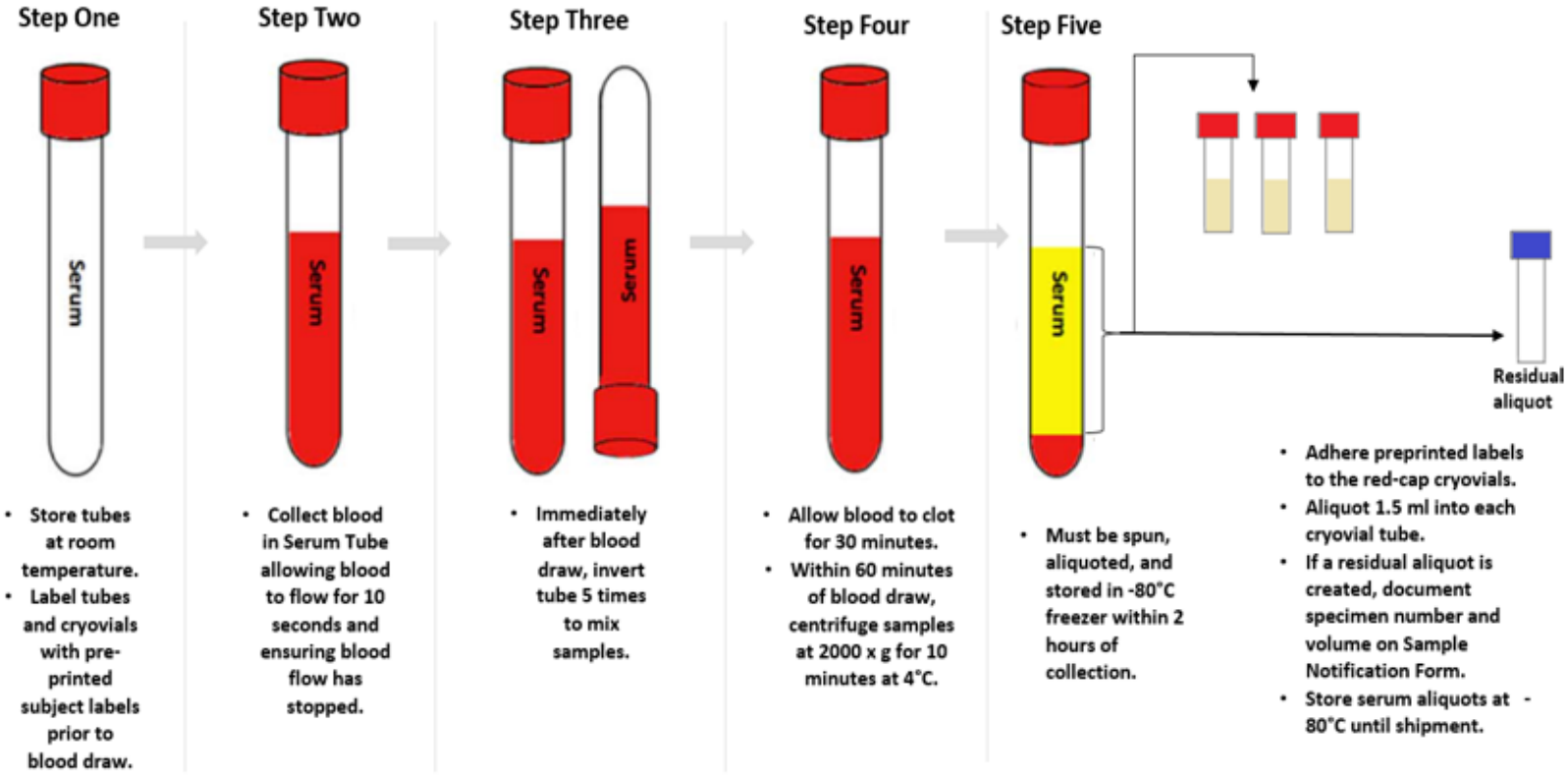
   a. Equivalent rpm for spin at 2000 x g
   b. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form Appendix B.
   c. Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
   d. Record time aliquoted on the Biological Sample Shipment and Notification Form.

8. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the wall. Using a disposable pipette, transfer serum into the pre-labeled cryovials with the red caps. Aliquot 1.5 ml per cryovial (total vials= up to 3 with 1.5 ml each and 1 residual with <1.5 ml). The Serum tube should yield, on average, 4- 5 ml of serum for a total of (3) 2.0 ml aliquot cryovial tubes per participant with 1.5 ml per cryovial tube. Be sure to only place **serum** in cryovials labeled with the "SERUM" label and red caps. If there is extra serum left, use 1 extra cryovial provided for another <1.5 ml aliquot of serum and label as appropriate. **If a residual aliquot (<1.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form (Appendix B same as source document N15).**




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

# Serum Preparation (10ml Red Top Tube)




*Ensure tubes are not expired prior to blood draw*

**Please be sure to compare the labels on each tube and cryovials to the Biological Sample Form included with each kit**

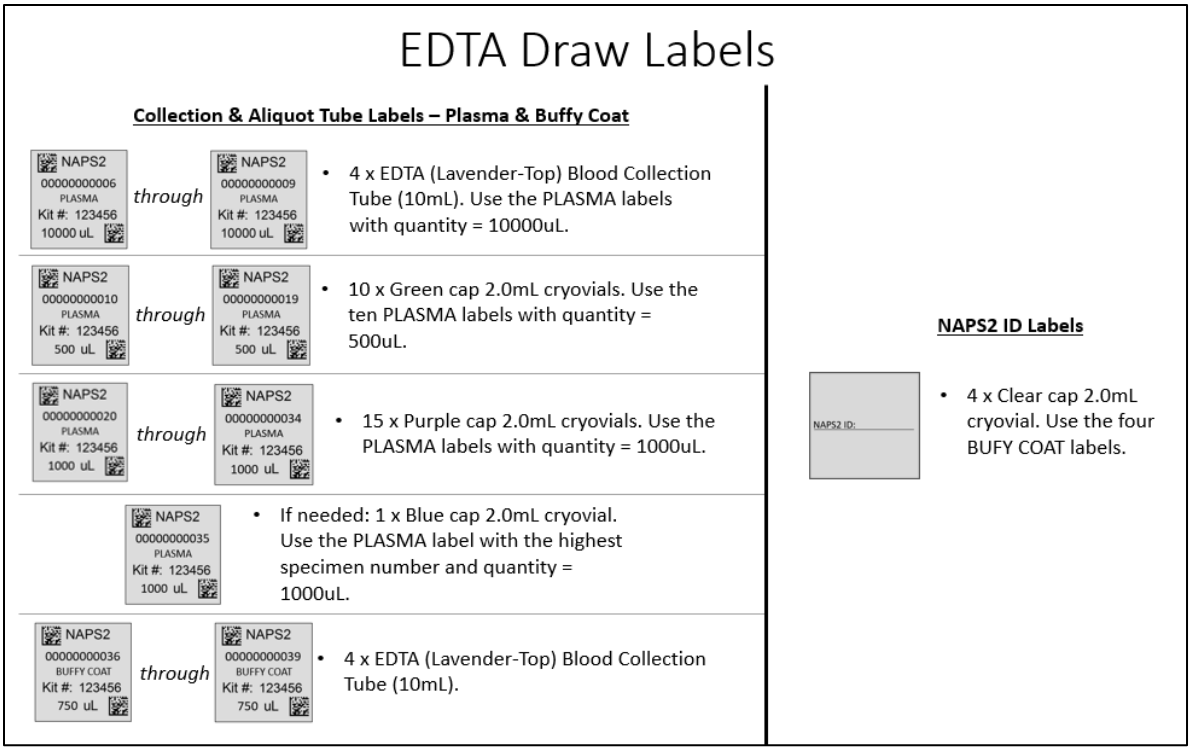
## 7.5 EDTA (Lavender-top) Blood Collection Tubes (10 ml) for Plasma and Buffy Coat

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

1. Store empty EDTA tubes at room temperature, 64°F - 77°F (18°C – 25°C) before use. Check expiration dates on all collection tubes before visit.

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

3. Locate and verify proper labeling for tubes.
    a. The 4 x EDTA (Lavender-top) Blood Collection Tubes (10 ml) should have:
        1. a handwritten ID label and
        2. a "**PLASMA**" Collection and Aliquot Tube Label. Please use the "**PLASMA**" Collection and Aliquot Tube Labels with quantity = 10000uL.
    b. The 10 x Green cap cryovials should each have a "**PLASMA**" Collection and Aliquot Tube Label. Please use the "**PLASMA**" Collection and Aliquot Tube Labels with quantity = 500uL.
    c. The 15 x Purple cap cryovials should each have a "**PLASMA**" Collection and Aliquot Tube Label. Please use the "**PLASMA**" Collection and Aliquot Tube Labels with quantity = 1000uL.
    d. If needed, the 1 x Blue cap cryovial should have a "**PLASMA**" Collection and Aliquot Tube Label. Please use the "**PLASMA**" Collection and Aliquot Tube Label with the highest specimen number and quantity = 1000uL.
    e. The 4 x clear cap cryovials should each have a "**BUFFY COAT**" Collection and Aliquot Tube Label.




4. Please ensure that aliquots are kept in numerical order (by specimen number) throughout the aliquoting and shipping process.

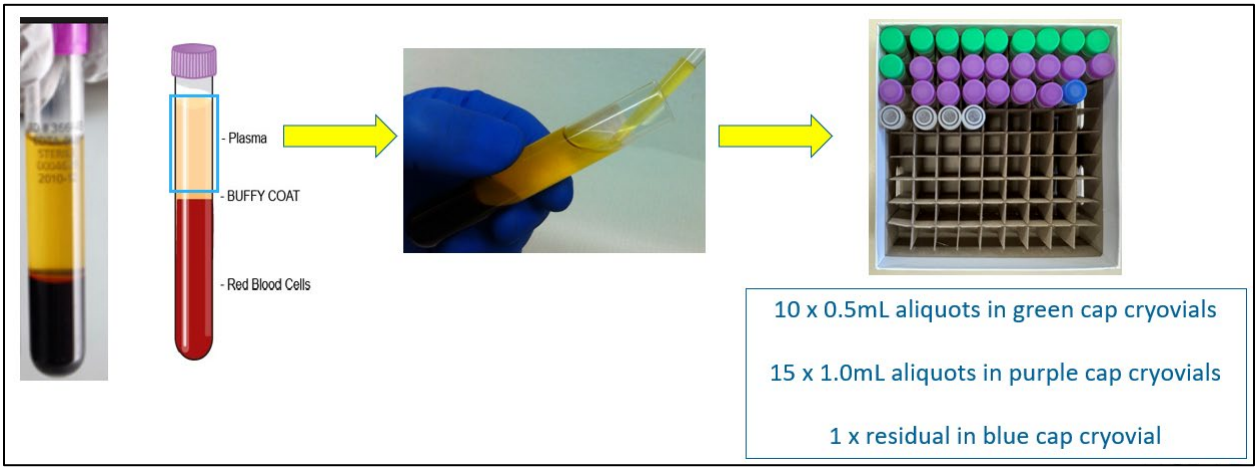
5. Using a blood collection set and a holder, collect blood into the **EDTA (Lavender-Top) Blood Collection Tubes (10 ml)** using your institution's recommended procedure for standard venipuncture technique.**The following techniques shall be used to prevent possible backflow:**
   a. Place donor's arm 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 the last tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

6. 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.

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

8. **CRITICAL STEP: Immediately after inverting the EDTA tubes, place on wet ice until centrifugation begins.**

    a. Preferably within 30 minutes of blood collection, centrifuge balanced tubes for 10 minutes at 2000 RCF (x g) at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM.**
    b. Equivalent rpm for spin at 2000 x g.
    c. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
    d. Plasma samples need to be spun, aliquoted, and placed upright in the freezer within 2 hours from the time of collection.
    e. Record time aliquoted on the Biological Sample and Shipment Notification Form.
9. Remove the plasma, being careful not to agitate the packed red blood cells at the bottom of the collection tube. Tilt the tube and placing the disposable pipette tip along the lower side of the wall without touching the pellet (buffy coat) so that plasma is not contaminated (see below). Transfer plasma from all four EDTA tubes into the 50 ml conical tube and gently invert 3 times. Aliquot 0.5 ml per green cap cryovial for the first 10 aliquots (10 vials with 0.5 ml each). Then aliquot 1.0 ml per purple cap cryovial using the remaining plasma. 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 the extra cryovial with blue cap provided for a residual aliquot of plasma. **If a residual aliquot is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**



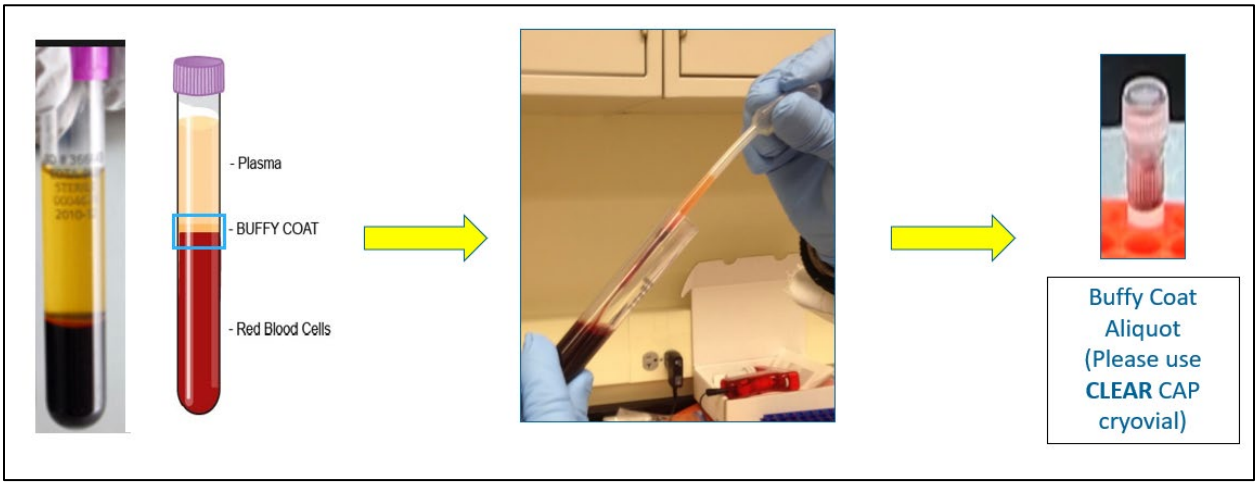

**NOTE: When pipetting plasma from the EDTA tubes into the 50 ml conical tube, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.**

10. Place the labeled cryovials in the cryobox and place on pelleted dry ice. Transfer to **-80°C Freezer when possible**. Store all samples upright at **-80°C until shipped** to

NCRAD on pelleted dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

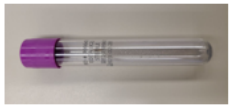
11. To aliquot buffy coats:

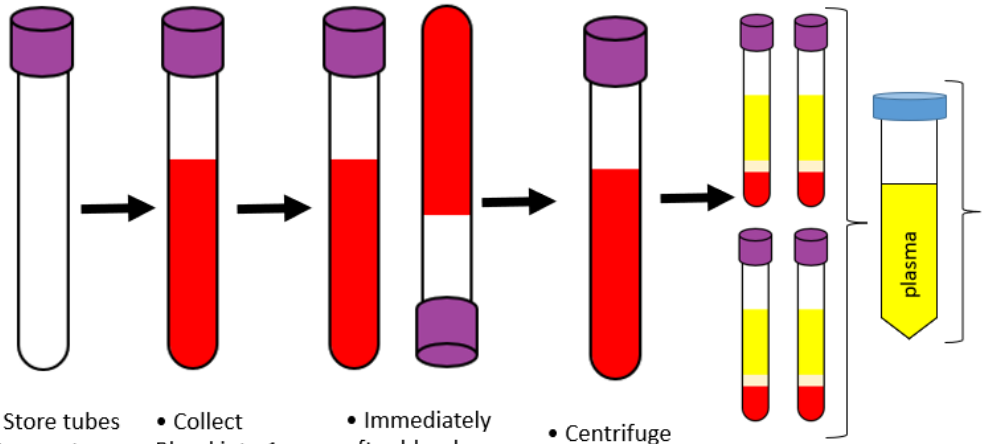
    a. After plasma has been removed from the EDTA (Lavender-Top) Blood Collection Tubes (10 ml), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) into labeled cryovials with clear caps using a disposable graduated micropipette. Aliquot each buffy coat into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




    b. Dispose of collection tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.
    c. Place the labeled cryovial in a cryobox and place on pelleted dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on pelleted dry ice. Sites have the option to keep 1-2 buffy coats from each visit locally.

## Plasma & Buffy Coat Preparation (EDTA Tube x 4)






- Store tubes at room temp
- Each tube should be labeled with Collection Tube and Site and PTID labels.

- Collect Blood into 1 EDTA tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped

- Immediately after blood draw, invert tube 8-10 times to mix sample.

- Centrifuge samples at 2000 x g for 10 minutes at 4°C

- 10 x 0.5 ml aliquots of plasma into green cap cryovials
- 15 x 1.0 ml aliquots of plasma into purple cap cryovials
- If residual aliquot is created, use the blue-capped cryovial and a "PLASMA" label. Document specimen number and volume on Sample Form
- Store plasma aliquots upright at -80°C until shipment to NCRAD

- Aliquot the buffy coat from each EDTA tube separately, into its own cryovial
- 4 x 1.0 ml aliquots of the buffy coat (may have some residual plasma and RBCs included) into the clear-capped cryovials.
- Store buffy coat aliquot upright at -80°C until shipment to NCRAD

*Ensure tubes are not expired prior to blood draw*

*Spin, aliquot, and freeze all plasma and buffy coat aliquots within 2 hours of collection*

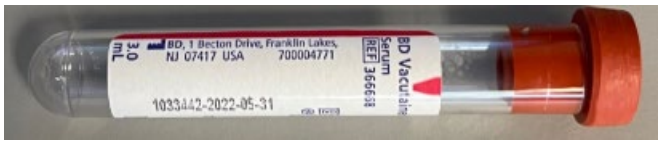
**Please be sure to compare the labels on each tube and cryovials to the Biological Sample Form included with each kit**

## 7.6 2.5 ml PAXgene™ Tube for RNA

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

1. Store empty 2.5ml PAXgene™ tubes at room temperature, 64°F - 77°F (18°C – 25°C) before use. Check expiration dates on all collection tubes before visit.

2. Locate and verify proper labeling for the Collection and Aliquot "**RNA**" Tube Label on the PAXgene™ tube prior to blood draw;




3. Using a blood collection set and a holder, collect blood into the **PAXgene™ RNA Tube** using your institution's recommended procedure for standard venipuncture technique.
   **The following techniques shall be used to prevent possible backflow:**

   a) Place participant's arm in a downward position.
   b) Hold tube in a vertical position, below the participant's arm during blood collection.
   c) Release tourniquet as soon as blood starts to flow into last 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 the tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The PAXgene™ RNA Tube with its vacuum is designed to draw 2.5 ml of blood into the tube.

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

6. Place the PAXgene™ RNA tube upright in a **WIRE** rack and transfer the PAXgene™ RNA tube to a **-80°C freezer.** Keep the **PAXgene™ RNA Tube in -80°C freezer** for storage until you ship on pelleted dry ice to NCRAD. No processing is required for this tube. **The single tube is to be shipped to NCRAD frozen, without processing at the collection site.** Keep the bubble wrap sleeve from the blood kit. It will be needed to protect the PAXgene tube when sending biospecimens to NCRAD.

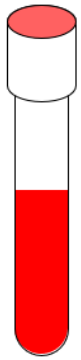
7. Complete remainder of the Biological Sample and Shipment Notification Form (Appendix B)

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


**Step One**

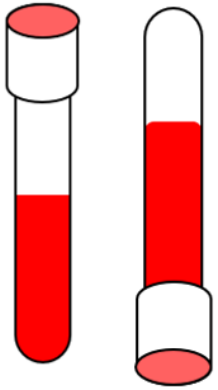
- Store tube at room temperature.
- Label tube with pre-printed labels prior to blood draw.

**Step Two**



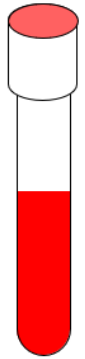
- Collect blood in $PAXgene^{TM}$ Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

**Step Three**



- Immediately after blood draw, invert tube 8-10 times to mix sample.

**Step Four**



- . Place the PAXgene™ RNA tube upright in a **WIRE** rack and transfer the PAXgene™ RNA tube to a **-80°C freezer.** Keep the **PAXgene™ RNA Tube in -80°C freezer** for storage until you ship on pelleted dry ice to NCRAD

**Please be sure to compare the labels on the tube to the Biological Sample Form included with each kit**

**Important Note**: Ensure tube is not expired prior to collection and processing of samples.

# 8.0 Cerebralspinal Fluid Collection and Processing

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

**CSF samples should be collected in the morning before breakfast and after an overnight fast when possible. Only water should be permitted past midnight, until lumbar puncture is completed.**

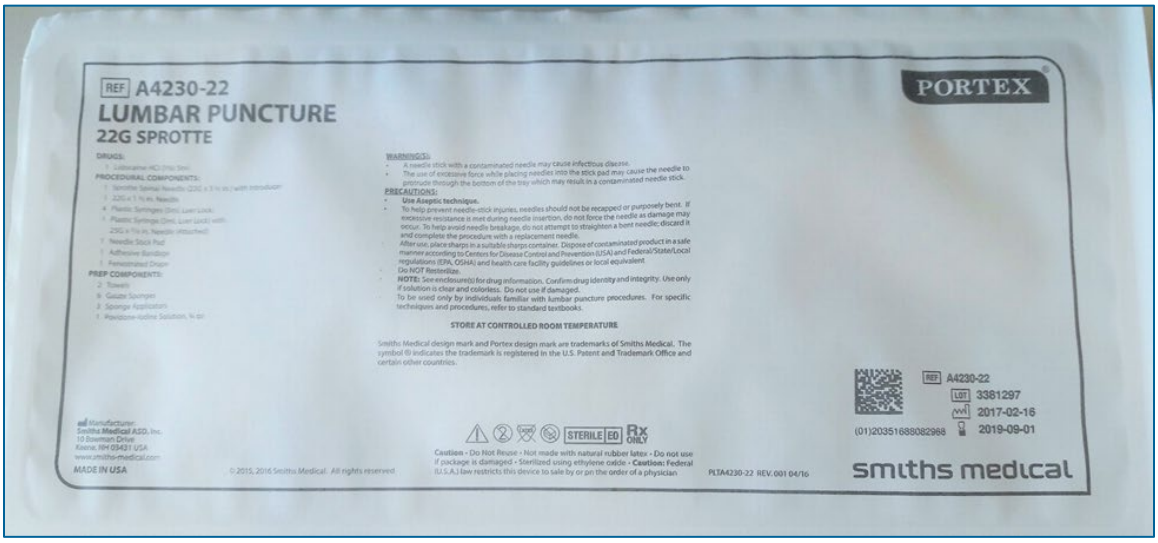
There are general guidelines to follow for CSF Collection.

- Begin by confirming participant consented to lumbar puncture (LP) before scheduling the procedure and again prior to performing procedure.
- Do NOT use any extension tubing due to the tendency of manufactured plastic tubing to bind beta amyloid peptides and other important AD biomarkers.
- If LP was attempted but unsuccessful in obtaining CSF, a second attempt under fluoroscopy (if deemed appropriate by site clinician) is allowed.
- If performing LP under fluoroscopy, site personnel should advise the participant that use of fluoroscopy (x-rays) involves exposure to radiation.
- Anti-platelet agent (e.g. aspirin) or anticoagulant medication (e.g. warfarin (Coumadin) and/or dabigatran (Pradaxa)) is at the discretion of the local site investigator. Site specific guidelines for discontinuing asprin and anticoagulant medication for a research lumbar puncture should be followed.
- Each study participant or a person designated to speak for them will be contacted by phone after the LP to confirm participant well-being and to query about any adverse events.
- Ensure you have at least two “Lumbar Puncture Tray Kits” and sufficient “CSF Supplemental Supply Kit” provisions on hand prior to scheduling an LP visit. Also ensure adequate site-provided supplies (see above), including pelleted dry ice.
- Check expiration dates on all supplies, especially lidocaine.

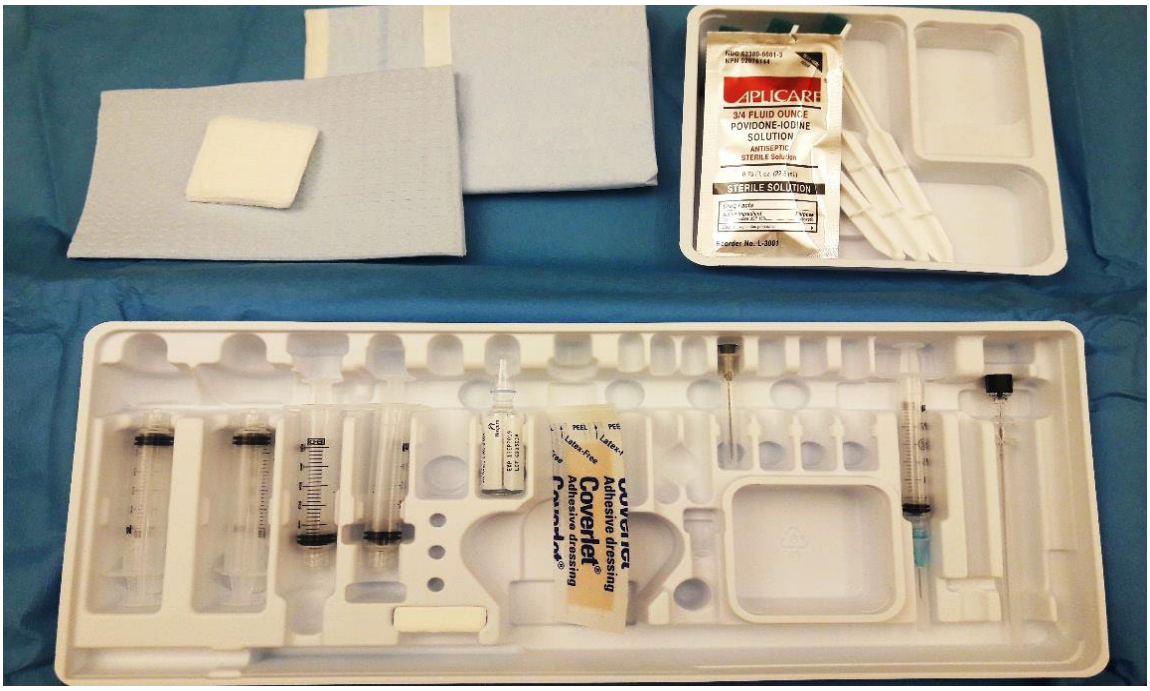
**Scheduling the LP**

- All LPs should be performed between 7am and 10am local time with the participant fasting. CSF amyloid levels can vary depending upon the time of day the sample is collected. It is important for the time of day of collection to remain consistent across study visits.
- The LP should be rescheduled if the participant does not feel well or is febrile.

**Lumbar Puncture Tray Kit Images**




Exterior of LP Tray provided by NCRAD which contains the 22 gauge Sprotte Needle with Introducer




Interior of LP Tray Provided by NCRAD.

## General CSF Collection Methods

The site is responsible for the CSF collection and follow up patient monitoring and care according to industry standards and with the appropriately licensed personnel. Sites must designate the method of CSF collection for data tracking purpose. It is recommended that CSF be obtained from participants in a sitting position. Alternate needles, positions or methods (e.g., use of fluoroscopy) should be noted on the CSF Sample and Shipment Notification Form.

**Collection of CSF**
**Discard first 1-2mls of CSF if blood tinged. If not blood tinged, collect first 1-2 mls of CSF into a 50ml conical tube and pipette into the yellow cap cryovial for local lab (if applicable). Collect 20-30 CSF total into the second 50ml conical tube.**

**Reminder:** If the CSF is blood-tinged, the first 1-2 ml of CSF should be discarded (or more if needed) to clear the blood before collecting the 20-30 ml for CSF analysis. 20 ml is the required MINIMUM for CSF biomarker analysis. If 20 ml is not obtained and provided to the NCRAD, document the reason for under-collection on the comments section of the CSF Sample and Shipment Notification Form (Appendix C, which is the same as source document N16).

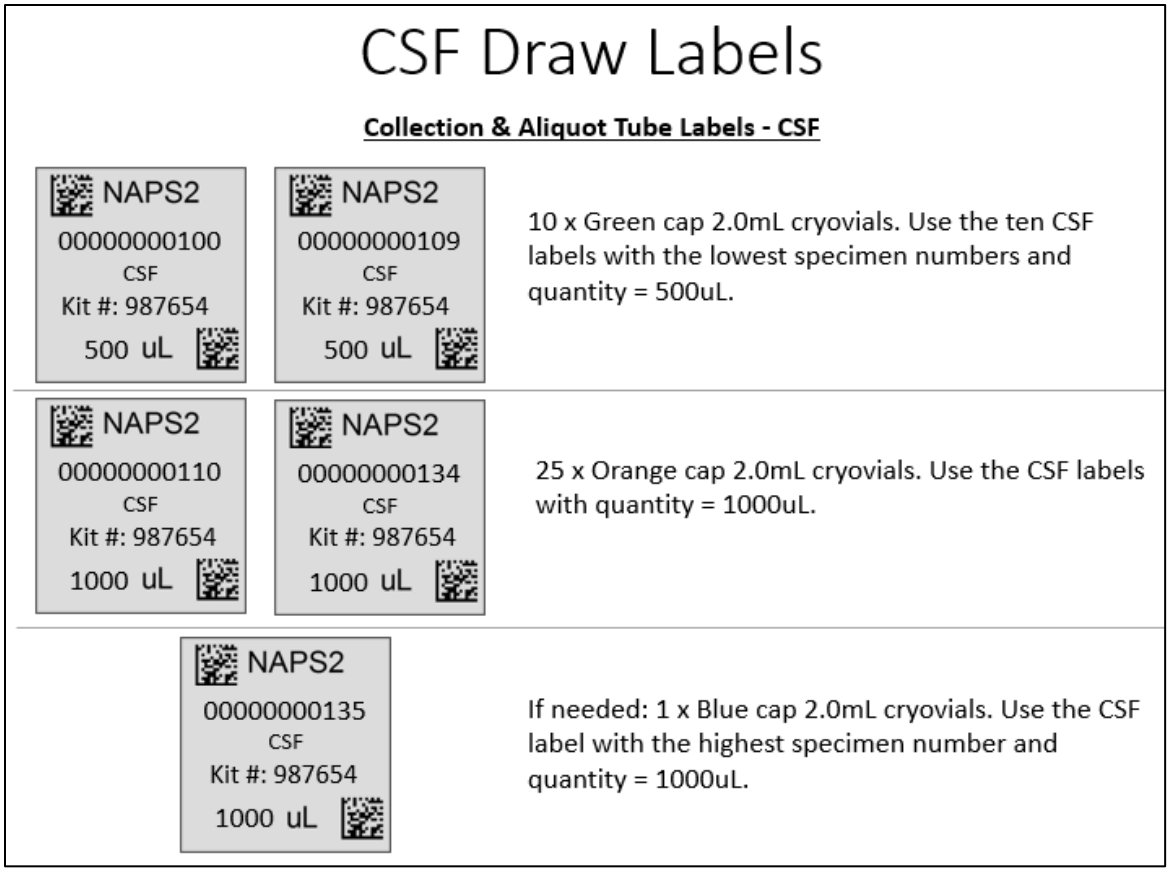
## Step by Step Summary of CSF Collection Procedure

Ensure all samples collected are appropriately labeled.

1. Print CSF Sample and Shipment Notification Form.

2. Confirm all supplies, including pelleted dry ice (~10 lbs) and wet ice, are available.

3. Label the (10) green cap cryovials, (25) orange cap cryovials, and (1) blue cap cryovial with provided NAPS2 CSF labels. Do **NOT** open and label the 50-ml tubes that will be kept sterile to collect the CSF.
    a. The (10) green cap cryovials should be labeled with the CSF labels with the 10 lowest specimen numbers and quantity = 500uL.
    b. The (25) orange cap cryovials should be labeled with the CSF labels with the next 10 sequential specimen numbers and quantity = 1000uL.
    c. If needed, the (1) blue cap cryovial should be labeled with the CSF label with the highest specimen number and quantity = 10000uL.
    d. Note: there is no provided label for the yellow cap cryovial.




4. Pre-cool the centrifuge and pre-cool all (36) labeled cryovials (yellow cryovials will not be labeled) on wet ice. Do **NOT** pre-cool the 50-ml tubes that will be kept sterile to collect the CSF.

5. Measure vitals (participant lying down).

6. Record the time of LP and associated information on the CSF Sample and Shipment Notification Form.

7. Collect 20-30 ml CSF (document method on CSF Sample and Shipment Notification Form) following these steps:

    a. Collect initial 1-2 ml (if bloody, collect CSF until cleared of blood) using the 50ml conical tube. If not bloody, transfer first 1-2ml into yellow cap cryovial for local lab.
    b. Collect an additional 20-30 ml CSF into the **UNLABELED-STERILE** 50-ml polypropylene tube from the "CSF Supply Kit". 20 ml is the required **MINIMUM. Collect no more than 30 ml total, including any discarded CSF and CSF kept for local lab.**
    c. Note: using **ASPIRATION IS A NAPS2 PROTOCOL VIOLATION** and must be reported as such. If aspiration must be used, it is advised to use **ONLY** the polypropylene syringes included in the "Lumbar Puncture Collection Kit" and

transfer **DIRECTLY** into the **UNLABELED-STERILE** 15-ml polypropylene tube from the "CSF Supply Kit". There are four 6 ml Luer lock polypropylene syringes in the "Lumbar Puncture Collection Kit." Note this on the CSF Sample and Shipment Notification Form.

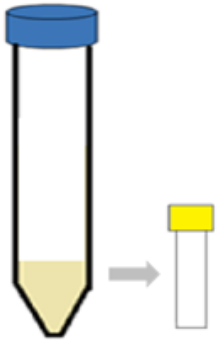
8. Process the CSF as follows:
    a. Affix NAPS2 ID label to outside of conical (keeping measuring lines visible) and place samples upright on wet ice prior to processing. Within 15 minutes of collection, centrifuge at 2000 x g for 10 min at 4°C to pellet any cellular debris.
    b. Being careful not to disturb the debris pellet, aliquot 0.5 ml of CSF into each green-cap cryovial for the first 10 aliquots (10 green cryovials with 0.5 ml each). Then aliquot 1.0 ml per orange-cap cryovial using the remaining CSF. If there is remaining CSF, create a residual aliquot by aliquoting into blue-cap cryovial. Document specimen number and volume on CSF Sample and Shipment Notification Form.
    c. Store CSF aliquots at -80°C and record time of freezing on CSF Sample and Shipment Notification Form.

# CSF Preparation (20-30 ml)

## Step One

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

## Step Two



- Collect initial 1-2 ml (if bloody, collect CSF until cleared of blood) into 50 ml conical tube.
- If not bloody, transfer 1-2 ml into the yellow-cap cryovial.
- Send to local lab for testing.

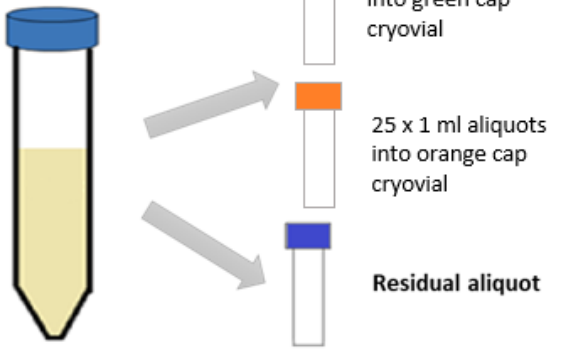
## Step Three

- Collect another 20-30 ml CSF into a new 50 ml sterile conical tube.

## Step Four

- Place sample upright on wet ice until centrifugation begins.
- Within 15 minutes of collection, centrifuge sample at 4°C for 10 minutes at 2000xg.

## Step Five




- Aliquot 0.5 ml into 10 x green cryovials.
- Aliquot 1 ml into 25 x orange-cap cryovials.
- If a residual aliquot is created, aliquot into blue-cap cryovial. Document specimen number and volume on CSF Sample Notification Form.
- Store CSF aliquots at -80°C until shipment.

# 9.0 Sample Redraws

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

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

**Redraws will be scheduled for samples submitted to NCRAD.**

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

1. If the biospecimens at a scheduled visit are partially collected:
    a. Attempt to process and submit any samples that were able to be collected during the visit.
    b. Document difficulties on the 'Biological Sample and Shipment Notification Form' prior to submission to NCRAD.
        i. Indicate blood draw difficulties at the bottom of the 'Biological Sample and Shipment Notification Form' within the "Notes" section.
        ii. Complete the 'Biological Sample and Shipment Notification Form' with tube volume approximations and number of aliquots created.
    c. Contact a NCRAD coordinator and alert them of the challenging blood draw.
2. If the biospecimens at a scheduled visit **are not** collected:
    a. Contact the NAPS2 Project Manager 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 re-draw visit as quickly as possible.

## 10.0 Sample Packaging and Shipping Instructions

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

| Sample Type | Collection Tube | Processing/ Aliquoting | Tubes to NCRAD | Ship |
|---|---|---|---|---|
| **Whole blood for isolation of Serum** | 1 x Plain Red-Top Serum Blood Collection Tube (10ml) | 1.5 ml serum aliquot per 2.0 cryovial (red cap).<br><br>Rresidual volume placed in 2.0 cryovial with blue cap. | Up to 4 | Frozen |
| **Whole blood for isolation of plasma and buffy coat** | 4 x EDTA (Lavender-Top) Blood Collection Tube (10 ml) | PLASMA:<br>10 x 0.5ml aliquots in 2.0ml green cap cryovials.<br><br>15 x 1.0ml aliquots in 2.0ml purple cap cryovials.<br><br>Rresidual volume placed in 2.0ml cryovial with blue cap. | Up to 26 | Frozen |
| | | BUFFY COAT:<br>Aliquot buffy coat from each (4) EDTA tube into its own 2.0ml clear cap cryovial | Up to 4* | Frozen |
| **Whole blood for RNA extraction** | 1x PAXgene™ Blood Collection Tube (2.5 ml) | N/A | 1 | Frozen |
| **CSF** | Sterile Containers (20-30 ml CSF) | 10 x 0.5ml CSF in the first 10 green cap cryovials.<br><br>25 x 1.0ml CSF in 2.0 orange cap cryovials.<br><br>Residual volume place in 2.0ml cryovial with blue cap.<br><br>1 x 1-2ml CSF for local lab placed in 2.0ml yellow cap cryovial. | Up to 36 | Frozen |

*Sites may elect to keep 1-2 buffy coats from each visit locally.

**Large Frozen Shipper:**

** 45 lbs of dry ice pellets

**AND**

Fits up to 4 x 81-cell cryoboxes **AND** 4 x 2.5ml PAXgene™ tubes.

**Small Frozen Shipper:**

**10 lbs of dry ice pellets

**AND**

Fits up to 2 x 81-cell cryoboxes **AND** 2 x 2.5ml PAXgene™ tubes.

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

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

## 10.1 Frozen Batch Shipping Instructions

**IMPORTANT:** FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY

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

- The primary receptacle (frozen cryovials) must be leak proof and must not contain more than 1L total.
- The secondary packaging (biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (within the cryovial box containing the frozen cryovials) and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.

- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the
- following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible Person
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373
  - ✓ Class 9 label including UN 1845, and net weight of dry ice contained


1. Hold packaged samples in -80C freezer until time of FedEx pick-up/drop-off.
2. A sample shipment to NCRAD should be initiated when a study site has **four (4) cryoboxes of samples**. If a participant has a cryobox with blood samples and a cryobox with CSF samples, this counts as two boxes towards the total of eight cryoboxes to batch ship.
3. Contact FedEx to confirm service is available and schedule package to be picked up.
4. Notify NCRAD of shipment by emailing NCRAD coordinators at alzstudy@iu.edu
5. Attach the Completed Biological Sample and Shipment Form to the email. If email is unavailable, please call NCRAD and do not ship until you have notified NCRAD coordinators of the shipment in advance.
6. Place all labeled and frozen plasma, and buffy coat aliquots in a cryobox.
7. If collecting CSF, place up to 36 CSF aliquots in a 81-slot cryobox. Place up to 4 serum aliquots, 26 plasma aliquots, and 4 buffy coats in a separate 81-slot cryobox. Label the outside of the cryoboxes with the kit number labels and place in a clear biohazard bag. If the participant has CSF and blood samples from same cycle, both cryoboxes and PAXGene tube can be placed in the same biohazard bag and shipped to NCRAD together. Do not remove absorbent material found in the bag. Place frozen PAXgene™ tube in provided bubble wrap tube sleeve, seal, and place in biohazard bag with cryobox. Seal biohazard bag according to the instructions on the bag.

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

11. Replace the lid of the Styrofoam container. Place the completed Biological Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape. Be sure to NOT completely seal the outer cardboard box with tape, as the pelleted dry ice needs to vent.
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.
d. Section 7, “Payment”, check third party and bill transportation costs to the NAPS2 study FedEx account number. Please contact NCRAD or the NAPS2 Study Team for this account number.

13. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
    a. Your name and return address
    b. Net weight of pelleted dry ice in kg (must match amount on the airbill)
    c. Consignee name and address:

NCRAD
IU 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 the package, taking care not to overlap labels.
15. Specimens should be sent to the address below via FedEx Priority Overnight. Frozen specimens should be sent Monday through Wednesday to avoid any potential shipping delays. FedEx does not replenish dry ice if shipments are delayed or held over the weekend.

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

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

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

- Remember to complete the Biological Sample and Shipment Notification (Appendix B), include a copy in your shipment AND notify the NCRAD Study Coordinator by email at alzstudy@iu.edu (include Fed Ex tracking number in email) IN ADVANCE to confirm the shipment.
- In addition to tracking and reconciliation of samples, the condition and 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 pelleted dry ice to avoid thawing in the shipment process.

## 11.0 Data Query and Reconcilliation

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.

[Insert other queries depending on data collection team]

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 REDCap 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

## 12.0 Appendices List

## Appendix A. Rate of Centrifuge Worksheet

# 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:

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

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**

alzstudy@iu.edu

## Appendix B. Biological Sample and Shipment Notification Form

NAPS CONSORTIUM For REM Sleep Behavior Disorder

### Biological Sample and Shipment Notification Form

NCRAD

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

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

*General Information:*

From: _____ Date: _____ Kit Barcode

Phone: _____ Email: _____

NAPS2 ID: _____ GUID ID: _____

Sex: M F Year of Birth: _____

Visit (circle one): Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5 Cycle 6 Cycle 7 Cycle 8

Select one: □ Case □ Control

Tracking #: _____ CSF Collected? Yes No

*Blood Collection:* Blood Collected (circle one): Yes No

1. Date Drawn: _____ [MMDDYYYY] 2. Time of Draw: 24 hour clock: _____ [HHMM]

3. Date subject last ate: _____ [MMDDYYYY] 4. Last time subject ate: 24 hour clock: _____ [HHMM]

*Blood Processing:*

**RNA (PAXgene Tube)**

Total volume of blood drawn into a 1 x 2.5mL PAXgene RNA tube: _____ mL

Date PAXgene RNA tube placed in -80°C freezer: _____

Time PAXgene RNA tube placed in -80°C freezer: 24 hour clock: _____ [HHMM]

**Serum (Red Top Tube)**

Time spin started: 24 hour clock: _____ [HHMM] Duration of centrifuge: _____ minutes

Temp of centrifuge: _____ °C Rate of centrifuge: _____ x g

Original volume drawn (1x10mL Serum tube): _____ mL

Time aliquoted: _____ [HHMM] Number of 1.5mL serum aliquots created: _____ x 1.5mL

If applicable, volume of residual serum aliquot (less than 1.5 mL) (Blue cap): _____ mL

If applicable, specimen number of residual serum aliquot (Last four digits): _____

Time aliquots placed in freezer: 24 hour clock: _____ [HHMM] Storage temperature of freezer: _____ °C

**Plasma & Buffy Coat (EDTA (Lavender Top) Tube - 10mL)**

Time spin started: 24 hour clock: _____ [HHMM] Duration of centrifuge: _____ minutes

Temp of centrifuge: _____ °C Rate of centrifuge: _____ x g

Original volume drawn (4x10mL EDTA tube):

EDTA #1: _____ mL EDTA #2: _____ mL EDTA #3: _____ mL EDTA #4: _____ mL Total Volume: _____ mL

Time aliquoted: _____ [HHMM]

Plasma

Number of 0.5mL plasma aliquots created (green cap): _____ x 0.5mL

Number of 1.0mL plasma aliquots created (purple cap): _____ x 1.0mL

If applicable, volume of residual serum aliquot (Blue cap): _____ mL

If applicable, specimen number of residual plasma aliquot (Last four digits): _____

Time aliquots placed in freezer: 24 hour clock: _____ [HHMM]

Buffy Coat

Buffy Coat aliquot #1 (last four digits): _____ Buffy Coat aliquot #2 (last four digits): _____

Buffy Coat aliquot #1 Volume: _____ mL Buffy Coat aliquot #2 Volume: _____ mL

Buffy Coat aliquot #3 (last four digits): _____ Buffy Coat aliquot #4 (last four digits): _____

Buffy Coat aliquot #3 Volume: _____ mL Buffy Coat aliquot #4 Volume: _____ mL

Time aliquots placed in freezer: 24 hour clock: _____ [HHMM] Storage temperature of freezer: _____ °C

Notes:

Ver: 02.2024

## Appendix C. CSF Sample and Shipment Notification Form

NAPS CONSORTIUM For REM Sleep Behavior Disorder

### CSF Sample and Shipment Notification Form

NCRAD

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

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

*General Information:*

From: _____ Date: _____ [MM/DD/YYYY]

Phone: _____ Email: _____

**Tracking #:** _____

*NAPS2 Participant Study Information:*

**NAPS2 ID:** _____ **GUID ID:** _____

**Sex (circle one):** Male Female **Year of Birth:** _____

**Select one:** □ Case □ Control

*Visit Information:*

**CSF Collected?** Yes No

Kit Barcode

**Gauge needle used for LP (circle one):** 22G 24 G

**Visit (circle one):** Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5 Cycle 6 Cycle 7 Cycle 8

**Collection Process:** Gravity Method Aspiration

(If aspiration method is used, it must be documented as a protocol violation)

*CSF Collection:*

1. Date of Collection: _____ [MMDDYYYY]
2. Time of Collection: 24 hour clock: _____ [HHMM]
3. Date subject last ate: _____ [MMDDYYYY]
4. Last time subject ate: 24 hour clock: _____ [HHMM]

*CSF Processing:*

Time Spint Started: 24 hour clock: _____ [HHMM]

Duration of Centrifuge: _____ minutes

Temperature of Centrifuge: _____ °C Rate of Centrifuge: _____ xg

Total Amount of CSF Collected: _____ mL

Time Aliquoted: _____ [HHMM]

Number of 0.5 mL CSF aliquots created (green cap): _____ x 0.5mL

Number of 1.0 mL CSF aliquots created (orange cap): _____ x 1.0mL

If applicable, volume of residual CSF aliquot (blue cap): _____ mL

If applicable, specimen number of residual CSF aliquot: _____

Time Frozen: _____ [HHMM] Storage Temperature of Freezer: _____ °C

*Notes:*

Ver: 02.2024

## Appendix D. Demographics Form

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

4. Compete legal given (first) name of participant at birth: _____
5. Complete additional (middle) name or names at birth: _____
6. Complete legal family (last) name of participant at birth:_____
7. Suffix:_____
8. Date of Birth:_____
9. Name of city/Municipality in which participant was born:_____