DUKE/UNC ADRC PROTOCOL UPDATE:

V03.2021

<table>
<thead>
<tr>
<th>Section</th>
<th>Change</th>
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<tbody>
<tr>
<td>8.1</td>
<td>Renamed “Frozen Packaging Instructions”</td>
</tr>
<tr>
<td>8.1.1</td>
<td>Renamed “NCRAD Packaging Instructions – Frozen Shipments”</td>
</tr>
<tr>
<td>8.2</td>
<td>Added “Frozen Shipping Instructions”</td>
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Duke University/University of North Carolina
Alzheimer’s Disease Research Center

in collaboration with the

National Centralized Repository for
Alzheimer’s Disease and Related Dementias

Biospecimen Collection, Processing, and Shipment Manual of Procedures

Version 03.2021
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# 1.0 Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetra-acetic Acid</td>
</tr>
<tr>
<td>IATA</td>
<td>International Air Transport Association</td>
</tr>
<tr>
<td>NCRAD</td>
<td>National Centralized Repository for Alzheimer’s Disease and Related Dementias</td>
</tr>
<tr>
<td>PHI</td>
<td>Protected Health Information</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cells</td>
</tr>
<tr>
<td>RCF</td>
<td>Relative Centrifugal Force</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions Per Minute</td>
</tr>
</tbody>
</table>
2.0 PURPOSE

The collection of biofluids is an important part of the Duke/UNC Alzheimer’s Disease Research Center (ADRC). The purpose of this manual is to provide study staff (PIs, study coordinators, phlebotomists) with instructions for collection and submission of biological samples for Duke/UNC ADRC study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

The following samples will be sent to NCRAD:

- Plasma
- Buffy Coat (DNA Extraction)
- 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 specimens provided to NCRAD for the Duke/UNC ADRC 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

Kaci Lacy, MPH, CCRP, Clinical Research Coordinator
Phone: 317-278-1170
Email: lacy@iu.edu

General NCRAD Contact Information
Phone: 1-800-526-2839
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: www.ncrad.org

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

For packing and shipment details of 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 **NCRAD Holiday Observations**

<table>
<thead>
<tr>
<th>Date</th>
<th>Holiday</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 1</td>
<td>New Year’s Day</td>
</tr>
<tr>
<td>3(^{rd}) Monday in January</td>
<td>Martin Luther King, Jr Day</td>
</tr>
<tr>
<td>4(^{th}) Monday in May</td>
<td>Memorial Day</td>
</tr>
<tr>
<td>July 4</td>
<td>Independence Day (observed)</td>
</tr>
<tr>
<td>1(^{st}) Monday in September</td>
<td>Labor Day</td>
</tr>
<tr>
<td>4(^{th}) Thursday in November</td>
<td>Thanksgiving</td>
</tr>
<tr>
<td>4(^{th}) Friday in November</td>
<td>Friday after Thanksgiving</td>
</tr>
<tr>
<td>December 25</td>
<td>Christmas Day</td>
</tr>
</tbody>
</table>

Please note that between December 24\(^{th}\) and January 2\(^{nd}\), Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2\(^{nd}\). 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 20\(^{th}\) 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](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 DUKE/UNC ADRC LABORATORY COLLECTION

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

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

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

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

In order to ship specimens, you must provide:

- Dry ice (approximately 45 lbs per shipment)

4.2 Biospecimens Sent to NCRAD
Samples are to be submitted according to the shipping methods outlined in Section 8.0. Guidelines for the processing, storage location, and timing of sample collection are listed in the tables below.

4.2.1 Biofluid Collection Schedule

<table>
<thead>
<tr>
<th>Biospecimen</th>
<th>Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>X</td>
</tr>
<tr>
<td>Buffy Coat (DNA)</td>
<td>X</td>
</tr>
<tr>
<td>CSF</td>
<td>X</td>
</tr>
</tbody>
</table>

Whole blood is collected in one 10 ml purple-top EDTA tube for shipment to NCRAD. The 10 ml EDTA and tube is processed locally into plasma and buffy coat fractions; they are then aliquoted, frozen at the study site, and shipped to NCRAD.
Consent forms must specify that any biological samples and de-identified clinical data may be shared with academic and/or industry collaborators through NCRAD. Recommended consent language can be found on the NCRAD website at: https://ncrad.org/recommended_consent_language.html. A copy of the consent form for each participant should be kept on file by the site investigator.

4.2.2 Biofluid Collection Chart

<table>
<thead>
<tr>
<th>Collection Tube</th>
<th>Specimen Type</th>
<th>Aliquot Volume</th>
<th>Total Number of Aliquots</th>
<th>Shipping Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 EDTA (Purple-Top) Blood Collection Tubes (10 ml)</td>
<td>Plasma</td>
<td>1.5 ml plasma aliquots</td>
<td>Up to 4</td>
<td>Frozen</td>
</tr>
<tr>
<td></td>
<td>Buffy Coat</td>
<td>~1.0 ml buffy coat aliquots</td>
<td>1</td>
<td>Frozen</td>
</tr>
<tr>
<td>Sterile Container</td>
<td>CSF</td>
<td>1.5 ml CSF aliquots</td>
<td>1</td>
<td>Frozen</td>
</tr>
</tbody>
</table>

5.0 SPECIMEN COLLECTION KITS, SHIPPING KITS, AND SUPPLIES

NCRAD will provide: 1) Sample collection kits for research specimens to be stored at NCRAD, the Supplemental Supply Kit, and the Frozen Shipment Kit and 2) clinical lab supplies (with the exception of dry ice and equipment supplies listed in Section 4.1). The provided materials include blood tubes, boxes for plasma, buffy coat, and CSF aliquots, as well as shipping labels to send materials to NCRAD. Kit number labels, Patient ID labels, collection tube labels, and cryovial labels will all be provided by NCRAD. Details regarding the kits are found in this Manual of Procedures. Collection tube and cryovial labels will be preprinted 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 NCRAD 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.
### Duke/UNC ADRC Blood Kit

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Duke/UNC ADRC Blood Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EDTA (purple-top) blood collection tube (10 ml)</td>
</tr>
<tr>
<td>3</td>
<td>Cryovial (2.0 ml) with purple cap</td>
</tr>
<tr>
<td>1</td>
<td>Cryovial (2.0 ml) with blue cap</td>
</tr>
<tr>
<td>1</td>
<td>Cryovial (2.0 ml) with gray cap</td>
</tr>
<tr>
<td>1</td>
<td>Preprinted Collection Tube Label</td>
</tr>
<tr>
<td>5</td>
<td>Preprinted Cryovial Label</td>
</tr>
<tr>
<td>3</td>
<td>Preprinted Kit Number Label</td>
</tr>
<tr>
<td>2</td>
<td>Label for handwritten Patient ID</td>
</tr>
</tbody>
</table>

### Duke/UNC ADRC CSF Kit

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Duke/UNC ADRC CSF Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cryovial (2.0 ml) with orange cap</td>
</tr>
<tr>
<td>1</td>
<td>Preprinted Cryovial Label</td>
</tr>
<tr>
<td>2</td>
<td>Preprinted Kit Number Label</td>
</tr>
</tbody>
</table>

### Duke/UNC ADRC Supplemental Supply Kit

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Duke/UNC ADRC Supplemental Supply Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>EDTA (purple-top) blood collection tube (10 ml)</td>
</tr>
<tr>
<td>6</td>
<td>Cryovial (2.0 ml) with purple cap</td>
</tr>
<tr>
<td>2</td>
<td>Cryovial (2.0 ml) with blue cap</td>
</tr>
<tr>
<td>2</td>
<td>Cryovial (2.0 ml) with gray cap</td>
</tr>
<tr>
<td>2</td>
<td>Cryovial (2.0 ml) with orange cap</td>
</tr>
<tr>
<td>5</td>
<td>Label for handwritten Patient ID</td>
</tr>
<tr>
<td>1</td>
<td>Cryovial box (holds up to 81 cryovials)</td>
</tr>
<tr>
<td>1</td>
<td>Large biohazard bag with absorbent sheet</td>
</tr>
<tr>
<td>1</td>
<td>Pre-printed airbill</td>
</tr>
<tr>
<td>1</td>
<td>Dry Ice Shipping Label, UN3373 Label, Fragile Label, Biohazard Label</td>
</tr>
</tbody>
</table>

### NCRAD Frozen Shipping Supply Kit

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Frozen Shipping Kit Components for Blood-Based Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cryovial box (holds up to 81 cryovials)</td>
</tr>
<tr>
<td>1</td>
<td>Plastic Biohazard bag with absorbent sheet (large)</td>
</tr>
<tr>
<td>1</td>
<td>UPS return airbill and pouch</td>
</tr>
<tr>
<td>1</td>
<td>Shipping box/Styrofoam container (small)</td>
</tr>
<tr>
<td>1</td>
<td>Warning label packet with dry ice sticker</td>
</tr>
<tr>
<td>Quantities</td>
<td>Items Available upon request within the NCRAD kit module</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------------------------------</td>
</tr>
<tr>
<td>By Request</td>
<td>EDTA (purple-top) blood collection tube (10 ml)</td>
</tr>
<tr>
<td>By Request</td>
<td>Cryovial box (holds up to 81 cryovials)</td>
</tr>
<tr>
<td>By Request</td>
<td>Cryovial (2.0 ml) with purple cap</td>
</tr>
<tr>
<td>By Request</td>
<td>Cryovial (2.0 ml) with blue cap</td>
</tr>
<tr>
<td>By Request</td>
<td>Cryovial (2.0 ml) with gray cap</td>
</tr>
<tr>
<td>By Request</td>
<td>Cryovial (2.0 ml) with orange cap</td>
</tr>
<tr>
<td>By Request</td>
<td>UPS return airbill</td>
</tr>
<tr>
<td>By Request</td>
<td>Shipping box/Styrofoam container (small)</td>
</tr>
<tr>
<td>By Request</td>
<td>Plastic biohazard bag with absorbent sheet (large)</td>
</tr>
<tr>
<td>By Request</td>
<td>Disposable graduated transfer pipette</td>
</tr>
<tr>
<td>By Request</td>
<td>Warning label packet</td>
</tr>
<tr>
<td>By Request</td>
<td>UN3373 label</td>
</tr>
<tr>
<td>By Request</td>
<td>Biohazard label</td>
</tr>
<tr>
<td>By Request</td>
<td>Dry ice shipping label</td>
</tr>
<tr>
<td>By Request</td>
<td>Fine Point Permanent Markers</td>
</tr>
<tr>
<td>By Request</td>
<td>Patient ID Labels</td>
</tr>
</tbody>
</table>

5.2 Kit Supply to Study Sites

Each site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits. Please go to: [kits.iu.edu/duke](http://kits.iu.edu/duke) to request additional kits and follow the prompts to request the desired supplies.

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

6.0 **Blood Collection and Processing Procedures**

6.1 Labeling Samples

***Important Note***

In order to ensure the highest quality samples are collected, it is essential to follow the specific collection 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.
**Label Type Summary**

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

**Kit Number Labels** tie together all specimens collected from one subject at one visit. They should be placed in the designated location on the Blood and CSF Sample and Shipment Notification Forms (Blood and CSF kits will have different Kit Numbers).

**Patient ID Labels** are used to document the individual’s unique Patient ID. Place one label on the blood collection tube.

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

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

**Important Note**

Each collection tube will contain two labels: the collection tube label and the Site and PTID 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 PTID label.
In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place cryovial labels on **ALL** 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 Patient ID label on the EDTA (purple-top) tube **BEFORE** sample collection. This label is 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.
6.2 Whole Blood Collection with 10 ml EDTA (Purple-Top) Tube for Plasma and Buffy Coat

1. Store empty EDTA tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use.

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

3. Place completed Patient ID Label and preprinted PLASMA collection tube label on the purple-top EDTA tube. Place preprinted PLASMA cryovial labels on the three 2 ml cryovial with purple caps and one 2 ml cryovial with blue cap (if necessary, for residual). Place preprinted BUFFY COAT cryovial label on the 2 ml cryovial with gray cap.

4. Using a blood collection set and a holder, collect blood into the EDTA (Purple-Top) Blood Collection Tube (10 ml) using your institution’s recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:
   a. Place participant’s arm in a downward position.
   b. Hold tube in a vertical position, below the participant’s arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
   a. If complications arise during the blood draw, please note the difficulties on the Biological Sample and Shipment Notification Form (Appendix B). Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.

6. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8-10 times.

7. Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.

8. Centrifuge balanced tubes for 10 minutes at 2000 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).
   a. Equivalent rpm for spin at 2000 x g
   b. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
   c. Record original volume drawn for each tube in spaces provided on the Biological Sample Shipment and Notification Form.
   d. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
   e. Record time aliquoted on the Biological Sample Shipment and Notification Form.

9. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall being careful not to agitate the packed red blood cells at the bottom of the collection tube.

10. Each EDTA tube should yield, on average, 4-5 ml of plasma. Aliquot 1.5 ml plasma per cryovial. Be sure to only place **plasma** in cryovials with purple caps and labeled with **PLASMA** labels. Place residual plasma (<1.5 ml) in the blue-capped cryovial. If a residual aliquot (<1.5 ml) is created, document the specimen number and volume on the Biological Sample and Shipment Notification Form.
11. Place the labeled cryovials in the 81 cell cryobox and place on dry ice. Transfer to -80°C Freezer when possible. Store all samples at -80°C until shipped to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample Shipment and Notification Form.

12. After plasma has been removed from the EDTA (Purple-Top) Blood Collection Tubes (10 ml), aliquot the buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) from one EDTA tube into a labeled, gray-capped cryovial using a micropipette. The buffy coat aliquot is expected to have a reddish color from the RBCs.

13. Dispose of collection tube with red blood cell pellet according to your site’s guidelines for disposing of biomedical waste.
14. Record the specimen number and volumes of the EDTA tube and corresponding buffy coat sample on the Biological Sample Shipment and Notification Form.

15. Place the labeled cryovials in the 81 cell cryobox and place on dry ice. You may store up to 9 subjects’ samples in the same cryobox as shown in the picture below. **Transfer to -80°C Freezer when possible.** Store all samples at -80°C until shipped to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

Nine subjects’ blood and CSF samples stored in same cryobox
Plasma and Buffy Coat Preparation EDTA Purple-Top Tube (10 ml)

Step One: Store tube at room temp. Label tube with Collection Tube and Site and PTID Labels.

Step Two: Collect blood into 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: Place thoroughly mixed tube on wet ice until centrifugation begins.

Step Five: Centrifuge sample at 2000 x g for 10 minutes at 4°C.

Step Six: 

- Plasma
- Buffy Coat
- RBCs

Step Seven: Label purple-capped cryovials with “PLASMA” labels.
- Aliquot 1.5 ml plasma into each cryovial.
- If residual aliquot is created, document specimen number and volume on Sample Form.
- Store plasma aliquots upright at -80°C until shipment to NCRAD.

Step Eight: Label gray-capped cryovials with “BUFFY COAT” labels.
- Using a clean transfer pipette, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat from each EDTA tube into its own cryovial.
- Store buffy coat aliquots upright at -80°C until shipment to NCRAD.
- Spin, aliquot, and freeze all plasma and buffy coat aliquots within 2 hours of collection.
7.0 CEREBROSPINAL FLUID COLLECTION AND PROCESSING

***Important Note***
CSF samples should be collected in the morning before breakfast and after an overnight fast. There should be a minimum 6-hour fast before collection of biomarker fluids and CSF. Only water is permitted until blood draws and the lumbar puncture are completed.

There are general guidelines to follow in regards to CSF Collection.

- Begin by confirming participant consented to lumbar puncture (LP) before scheduling the procedure and again prior to performing procedure.
- If LP and PET scan are done on the same day, LP should be completed prior to the PET scan; otherwise there should be at least 12 hours between LP and PET scan.
- LP should occur after, or a minimum of 72 hours prior, to an MRI scan.
- 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.
- LP under fluoroscopy is permitted, if needed. Site personnel should advise the participant that use of fluoroscopy (x-rays) involves exposure to radiation.
- Participants taking an anti-platelet agent (e.g. aspirin) may, at the discretion of the site clinician, be discontinued from that agent for a period of time prior to lumbar puncture and/or continue off agent for a period of time post LP. Participants who are taking anticoagulants (e.g. warfarin (Coumadin) and/or dabigatran (Pradaxa)) may not undergo an LP and are not suitable to participate in this study.
- Each study participant or a person designated to speak for them will be contacted by phone one day after the LP to confirm participant well-being and to query about any adverse events.
- Identify a physician (e.g., anesthesiologist) able to perform a blood patch for any participant who experiences a post lumbar puncture headache. Find out ahead of time who to call to schedule and perform a blood patch at your center, should the need arise. Ensure billing procedures are in place ahead of time.
- Ensure you have at least two lumbar puncture trays 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.
7.1 Scheduling the LP

All LPs should be performed in the morning if possible. Availability of staff and facilities for next day blood patch should be considered when scheduling LPs. 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.

7.2 Performing the LP

The recommended position is sitting with curved back and head down. For comfort, a stool may be used to prop up the feet and legs. The same position should be used at follow-up LPs. It is critical to try to optimize positioning, and usually requires an assistant. Other positions and needles are allowed (e.g., when using fluoroscopy) but this should be recorded on the CSF Sample and Shipment Notification Form. A pillow may be placed under the head for comfort.

On the bedside table nearest where the person performing the lumbar puncture will sit, place a pair of sterile gloves (in their packaging) and a blue pad. Remove the contents of the lumbar puncture tray from the outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything wrapped until the person performing the lumbar puncture is seated.

Feel the outside of the lumbar puncture kit (still wrapped up) to determine which end contains the spongy swabs. Turn this end toward the person performing the lumbar puncture and begin un-wrapping the kit.

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 outside of the wrapper touches any part of the inside of the wrapper, throw the kit away and start over.

If you are in any doubt as to whether the inside of the wrapper has been touched, throw the kit away and start over.

Cleaning the Lumbar Puncture Site
The lumbar puncture site is cleaned with Povidone-Iodine Topical Solution according to best standard medical practices.

Once the kit is successfully unwrapped, open the bottle of Povidone-Iodine Topical Solution somewhere away from the kit. Use an alcohol swab to remove any loose chunks of dried material off of the bottle top. You don’t want anything to fall onto the open and sterile lumbar puncture kit. Pour enough Povidone-Iodine Topical Solution into the prep well to cover the bottom, about ¼ inch deep.

Maintaining the Sterile Field
An important aspect of assisting with a successful lumbar puncture is keeping the field sterile. If there are a number of staff members in the room, please be sure they do not accidentally contaminate the sterile field. Once the person performing the lumbar puncture has donned sterile gloves, additional help may be needed to obtain or un-wrap any new tubes, needles, or supplies.

Unwrapping the Sterile 15 ml Conical Tube
Note that the 15 ml Falcon tubes into which CSF is collected and transferred come individually wrapped and are sterile inside and out. These wrappers should be peeled open by an assistant (not touching the tube) and the tube carefully dropped onto the LP tray or elsewhere in the sterile field in a manner that avoids contamination. Any additional needles or other individually-wrapped sterile items can be handled the same way.

Do not drop any packaging onto the tray or sterile field.

Do not let the item touch the outside of the packaging on its way to the tray.

Lidocaine, Syringe with Needle, Gauze Pads
Anesthesia is usually achieved within 2 minutes after injecting the lidocaine. Occasionally, the person performing the lumbar puncture will need to use more lidocaine to numb up a particular spot, or they may need to move to another spot entirely.

Next, hold the lidocaine bottle upside down and at a slight angle toward the person performing the lumbar puncture so that they can plunge the needle into
the bottle and extract some lidocaine without touching you or the bottle. Use two hands to stabilize the bottle. If the person performing the LP requires additional sterile gauze, open the gauze pad the same way as the syringe and needle, by holding open the package so the person performing the lumbar puncture can grab the gauze without touching you or the package.

**General CSF Collection Methods**

LPs for CSF collection should be performed using a small caliber atraumatic needle. CSF should be obtained via gravity flow using the 25 gauge Sprotte needle, although aspiration through this or other needles is allowable. Prior approval from the Clinical Core is required before the aspiration method can be utilized. 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 by Gravity**

After the spinal needle enters the L3-4 or adjacent intrathecal space and the stylet is withdrawn, CSF should flow freely. **Discard first 1-2 ml of CSF if blood tinged. If not blood tinged, collect first 1-2 ml of CSF into a 15 ml conical tube and pipette into a cryovial for local lab testing. Collect CSF into 15 ml Falcon 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 remainder of the CSF for analysis.

**Washcloths, Band-Aids, and Clean Up**

After the person performing the lumbar puncture collects the last of the CSF, remove the needle and introducer and wash the Povidone-Iodine Topical Solution off the participant. A warm, wet washcloth can be used. A Band-Aid should be applied to the puncture site. The participant should lie flat for 30-60 minutes. Next, discard the LP kit following local guidelines, and dispose of sharp components in an appropriate sharps container.

**Suggested management of post-lumbar puncture headache**

Classic post-lumbar puncture (low pressure) headache typically begins 24-48 hours after dural puncture, and the headache is worse when the participant is upright (sits or stands) and improves when the participant is recumbent with the head no higher than the spinal cord.

Safety and comfort of the LP is maximized by the use of atraumatic needles. The protocol requires use of a 25 gauge Sprotte needle. Lumbar puncture is a standard procedure for collection of CSF but may be associated with pain during
the performance of the procedure, comparable to the level of pain experienced during a blood draw. This is usually temporary and confined to the lower back. A persistent low-pressure headache may develop after lumbar puncture, probably due to leakage of CSF. If a post-LP headache persists it may need additional treatment, e.g. with fluids and analgesics. Uncommonly, a blood patch (injection of some of the participant’s blood to patch the CSF leak) may be needed.

Prevention: Use of a small gauge and atraumatic needle with careful technique are helpful in preventing post-lumbar puncture headache. Having the participant refrain from exercise or strenuous activities (especially heavy lifting) and staying well-hydrated for 24 hours after the LP may minimize the chance of a lumbar puncture headache.

Treatment of headache after a lumbar puncture:

- Limit physical activity as much as possible for at least 24 hours post-procedure.
- Increase oral fluid intake. Caffeine may be helpful.
- Routine analgesics such as acetaminophen may be used.

Post-lumbar puncture headache often resolves with the above treatment. If the headache persists after 24 hours of this management, it will likely require a blood patch. A blood patch typically relieves the headache instantly.

7.3 Step by Step Summary of CSF Collection Procedure

1. Print CSF Sample and Shipment Notification Form (Appendix C).

2. Confirm all supplies are available.

3. Label the one orange-capped cryovial for NCRAD with provided CSF cryovial label. Do NOT open and label the 15 ml Falcon tube that will be kept sterile to collect the CSF.

4. Pre-cool the centrifuge and pre-cool all cryovials on wet ice. Do NOT pre-cool the 15 ml Falcon tube 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 CSF at the L3/L4 position (or adjacent position) using a 25 gauge Sprotte spinal needle via gravity flow with participant in upright position (or...
document alternate 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 15 ml Falcon tube. If not bloody, transfer first 1-2 ml into cryovial for local lab.
b. Collect the rest of the CSF into the unlabeled and sterile 15 ml Falcon tube.
c. If using aspiration, use **ONLY** polypropylene syringes and transfer directly into the unlabeled and sterile 15 ml Falcon tube.

8. As one person takes the immediate post procedure vital signs, a second person should process the CSF as follows:

a. Place samples upright on wet ice and ensure samples are kept on wet ice for the entire time prior to processing. Preferably within 15 minutes of collection, centrifuge briefly at low speed (2000 x g, 10 min, 4°C) to pellet any cellular debris.
b. Using a clean transfer pipette, aliquot 1.5 ml into the orange-capped cryovial for shipment to NCRAD. Aliquot remaining CSF into cryovials for storage at site.
c. Within 2 hours of CSF collection, samples need to be spun, aliquoted and in the freezer. Store CSF aliquots at -80°C until shipment. Record time of freezing on CSF Sample and Shipment Notification Form.

9. Provide food and drink to participant (participant may lay flat to minimize the chance of a post-LP headache).

10. Place the labeled cryovials in the 81 cell cryobox and place on dry ice. Transfer to -80°C Freezer when possible. Store all samples at -80°C until shipped to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

Nine subjects’ blood and CSF samples stored in same cryobox
CSF Preparation

Step One
- Label orange-capped cryovial with pre-printed specimen label prior to collection.
- Pre-chill all cryovials on wet ice.

Step Two
- Collect initial 1-2 ml CSF for local lab testing.
- If bloody, collect CSF until cleared of blood.
- If not bloody, transfer into a separate cryovial. Send to local lab for cell count.

Step Three
- Collect CSF into 15 ml Falcon tube.

Step Four
- Place samples upright on wet ice until centrifugation begins.

Step Five
- Within 15 mins of collection, centrifuge sample at 4°C at 2000 x g for 10 minutes.

Step Six
- Using a clean transfer pipette, aliquot 1.5 ml into the orange-capped cryovial for NCRAD.
- Aliquot remaining CSF into cryovials for storage at site
- Store CSF aliquots upright at -80°C until shipment.
- **Spin, aliquot, and freeze aliquots within 2 hours of collection.**

To NCRAD
Store at site
8.0 Packaging & Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If you have difficulty finding biospecimen shipping training, please notify a NCRAD coordinator.

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 frozen samples are packed with sufficient amounts of dry ice to avoid thawing in the shipment process.

8.1 Frozen Packaging Instructions

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

***Important Note***
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.

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 Instructions – Frozen Shipments

1. Contact UPS 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:
   a. Completed Sample Form (Appendix B and Appendix C) to the email notification (email NCRAD coordinator prior to shipment to receive sample form).
   b. If email is unavailable please call NCRAD at 1-800-526-2839 and do not ship until you’ve contacted and notified NCRAD coordinators about the shipment in advance.

3. Place the cryovial boxes containing frozen samples into a biohazard bag.

4. As the cryovial box is placed in the plastic biohazard bag, do NOT remove the absorbent material found in the bag. Seal according to the instructions on the bag (shown on next page).

---

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

- The primary receptacle (cryovial) must be leak proof and must not contain more than 1L total.
- The secondary packaging (biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - Sender’s name and address
  - Recipient’s name and address
  - Responsible Person
  - The words “Biological Substance, Category B”
  - UN3373
  - UPS Dry Ice label and net weight of dry ice contained
5. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam shipping container.

6. Place the biohazard bags into the provided Styrofoam-lined shipping container on top of the dry ice. The frozen shipper can hold up to four 81 cell cryoboxes in biohazard bags. Please ensure that cryovial boxes are placed so the cryovials are upright in the shipping container.

7. Fully cover the biohazard bags containing the cryovial boxes tubes with approximately 2 inches of dry ice.

8. After the samples have been placed into the shipping container, fill the inner Styrofoam with plenty of dry ice pellets to ensure the frozen state of the specimens during transit.

9. The inner Styrofoam shipping container must contain approximately 10 lbs (or 4.5 kg) 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.

10. Replace the lid on the Styrofoam carton. Place the completed Blood 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.

11. Complete the UPS Dry Ice Label with the following information:
   a. Net weight of dry ice in kg (must match amount on the airbill)
b. Do not cover any part of this label with other stickers, including preprinted address labels.

12. Apply all provided warning labels and the preprinted UPS return airbill to the outside of package, taking care not to overlap labels.

***Important Note***
Complete the required fields on the UPS Dry Ice label or UPS may reject or return your package.

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

14. Specimens should be sent to the below address via UPS Next Day Air. Frozen shipments should be sent Monday through Wednesday to avoid shipping delays on Thursday or Friday.

   NCRAD
   Indiana University School of Medicine
   351 W. 10th St. TK-217
   Indianapolis, IN 46202

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

8.2 Frozen Shipping Instructions

1. Log into the ShipExec Thin Client at kits.iu.edu/UPS.
   a. If a new user or contact needs access, please reach out to your study contact for access.

2. Click “Shipping” at the top of the page and select “Shipping and Rating”.

3. Select your study from the “Study Group” drop down on the right side of the main screen. Choosing your study will automatically filter the address book to only addresses within this study.
4. Click on the magnifying glass icon in the “Ship From” section to search for your shipping address.

   a. Search by Company (site), Contact (name), or Address 1 (first line of your site’s street address). Click Search.
   b. Click Select to the left of the correct contact information.

5. Verify that both the shipping information AND study reference are correct for this shipment.
   a. If wrong study contact or study reference, click Reset in the bottom right of the screen to research for the correct information.

6. Enter Package Information
   a. Frozen shipments
      i. Enter the total weight of your package in the “Weight” field.
      ii. Enter the dry ice weight in the “Dry Ice Weight” field.
      iii. If the “Dry Ice Weight” field is higher than the “Weight” field, you will receive an error message after clicking Ship and need to reenter these values.
   b. Click Ship in the bottom right of the page when complete.

7. If your site does not already have a daily UPS pickup, you can schedule one here.
a. Click the blue Pickup Request button. Enter the earliest pickup time and latest pickup time in 24-hr format.
b. Give a name & phone number of someone who the UPS driver can call if having issues finding the package
c. Give the Floor and Room Number (if needed) to be as descriptive as possible where this package needs to be picked up from. Click Save.

8. Print the airbill that is automatically downloaded.
   a. To reprint airbill, click History at the top left of the page.
      i. Shipments created from the user that day will automatically populate. If shipments from a previous day need to be located, search by ship date.
      ii. Locate the correct shipment, and click on the printer icon to the left of the tracking number under “Action” to reprint the airbill
      iii. Click print icon on right side of the tracking number line.

9. Fold airbill, and place inside plastic UPS sleeve.

10. Peel the back off of the UPS sleeve, and stick the sleeve to the package.

9.0 DATA QUERIES AND RECONCILIATION

Sample and Shipment Notification forms must be completed on the day that samples are collected or before sample shipment because they include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses. Additional discrepancies may be sent directly to the Center staff to reconcile.

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

- 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 Blood Sample and Shipment Notification Form.

10.0 APPENDICES

Appendix A: Rate of Centrifuge Worksheet
Appendix B: Blood Sample and Shipment Notification Form
Appendix C: CSF Sample and Shipment Notification Form
Appendix A: Rate of Centrifuge Worksheet

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

Submitter Information
Name: __________________________ Site: __________________________
Submitter e-mail: __________________________

Centrifuge Information
Please answer the following questions about your centrifuge.

Centrifuge Type
Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

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

Calculating RPM from G-Force:

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

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

Comments:

Please send this form to NCRAD Study Coordinator
317-321-2003 (Fax) alzstudy@iu.edu
# Appendix B: Blood 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

### From:____________________________________  
**UPS tracking #:________________________________**

**Phone:**___________________________________  
**Email:**______________________________________

**Study:** Duke/UNC ADRC

**Patient ID:** ________________________________

**Sex:** [ ] M [ ] F  
**Year of Birth:** ____________

### Blood Collection:

<table>
<thead>
<tr>
<th>Date of Draw: [MMDDYY]</th>
<th>Time of Draw: [HHMM]</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Date subject last ate: [MMDDYY]</th>
<th>Time subject last ate: [HHMM]</th>
</tr>
</thead>
</table>

### Blood Processing: Plasma & Buffy Coat (EDTA Tube)

| EDTA specimen number  
_Last four digits:_ | Original blood volume of EDTA: ___ mL |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time spin started: <em>[HHMM]</em></td>
<td>Duration of centrifuge: ___ mins</td>
</tr>
<tr>
<td>Temp of centrifuge: <em>[°C]</em></td>
<td>Rate of centrifuge: ___ x g</td>
</tr>
<tr>
<td>Time aliquoted: <em>[HHMM]</em></td>
<td>Number of 1.5 mL plasma aliquots created (purple cap, up to 3): ___</td>
</tr>
<tr>
<td>If applicable, volume of residual plasma aliquot (less than 1.5 mL in blue cap): ___ mL</td>
<td>If applicable, specimen number of residual plasma aliquot (Last four digits): ___</td>
</tr>
</tbody>
</table>
| Buffy coat specimen number  
_Last four digits:_ | Buffy coat volume: ___ mL |
| Time aliquots frozen: _[HHMM]_ | Storage temperature of freezer: ___ °C |

### Notes:

__________________________________________________________________________________________
### CSF Sample and Shipment Notification Form

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

<table>
<thead>
<tr>
<th>From: ___________________________</th>
<th>UPS tracking #: ___________________________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phone: __________________________</td>
<td>Email: ________________________________</td>
</tr>
</tbody>
</table>

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

**Study:** Duke/UNC ADRC

<table>
<thead>
<tr>
<th>Patient ID: ___________________________</th>
<th>Kit barcode: ___________________________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: □M  □F  Year of Birth: ___________</td>
<td></td>
</tr>
</tbody>
</table>

**CSF Collection:**

<table>
<thead>
<tr>
<th>Date Drawn: _____________ [MMDDYY]</th>
<th>Time of Draw: ____________ [HHMM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date subject last ate: ___________ [MMDDYY]</td>
<td>Time subject last ate: ______ [HHMM]</td>
</tr>
</tbody>
</table>

Collection process: □ Gravitational  □ Pull

**CSF Processing:**

<table>
<thead>
<tr>
<th>Time spin started: __________ [HHMM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of centrifuge: ______ minutes</td>
</tr>
<tr>
<td>Temp of centrifuge: ______ °C</td>
</tr>
<tr>
<td>Rate of centrifuge: ______ x g</td>
</tr>
<tr>
<td>Total amount of CSF collected (mL): ______ ml</td>
</tr>
<tr>
<td>Time aliquoted: ______ [HHMM]</td>
</tr>
<tr>
<td># of 1.5 mL CSF aliquots created: (Orange-capped cryovial) ______</td>
</tr>
<tr>
<td>Time frozen: ______ [HHMM]</td>
</tr>
<tr>
<td>Storage temperature of freezer: ______ °C</td>
</tr>
</tbody>
</table>

**Notes:** ________________________________________________________________