# ADDS MANUAL OF PROCEDURES UPDATE:

## V06.2019

<table>
<thead>
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<th>Section</th>
<th>Change</th>
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Alzheimer’s Disease in Down Syndrome (ADDS)

in collaboration with

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

Blood-Based Biospecimens

Manual of Procedures

Version 4.2

June, 2019
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1.0 **ABBREVIATIONS**

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC-DS</td>
<td>Alzheimer’s Biomarker Consortium – Down Syndrome</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
</tr>
<tr>
<td>ADDS</td>
<td>Alzheimer’s Disease in Down Syndrome</td>
</tr>
<tr>
<td>CDCC</td>
<td>Columbia Data Coordinating Center</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DS</td>
<td>Down Syndrome</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>IUGB</td>
<td>Indiana University Genetics Biobank</td>
</tr>
<tr>
<td>NiAD</td>
<td>Neurodegeneration in Aging Down Syndrome: A Longitudinal Study of Cognition and Biomarkers of Alzheimer’s Disease</td>
</tr>
<tr>
<td>NCRAD</td>
<td>National Centralized Repository for Alzheimer’s Disease and Related Dementias</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>
<tr>
<td>SST</td>
<td>Serum Separator Tube</td>
</tr>
</tbody>
</table>
2.0 **PURPOSE**

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

- Identify blood-based and genetic biomarkers associated with the transition from normal aging to mild cognitive impairment to clinical dementia in individuals with DS
- Understand biomarker relationships and the pathways implicated in AD pathogenesis and develop a model for predicting risk.
- Set a foundation for an efficient transition from this biomarker study to a therapeutic trial to combat AD in DS augmented by biomarker outcomes

The purpose of this manual is to provide study staff (PIs, study coordinators, phlebotomists) at the various study sites with instructions for collection and submission of blood-based biological samples ADDS study visits. It includes instructions for blood-based biospecimen submission to NCRAD located in Indianapolis at Indiana University.

*The following samples will be sent to NCRAD:*

- Serum
- Plasma
- Buffy Coat (DNA extraction)

*Additional samples collected but not shipped to NCRAD:*

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

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

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

3.1 **NCRAD Contacts**

*Tatiana Foroud, PhD, NCRAD Leader*
Phone: 317-274-2218

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

*Colleen Mitchell, Laboratory Manager*
Phone: 317-278-9016
Email: mitchecm@iu.edu

**General NCRAD Contact Information**
Phone: 1-800-526-2839
Fax: 317-278-1100
Email: alzstudy@iu.edu
Website: [www.ncrad.org](http://www.ncrad.org)
ADDS-NiAD Study Specific Webpage:
[https://www.ncrad.org/resource_adds_niad.html](https://www.ncrad.org/resource_adds_niad.html)

*Kristi Wilmes, MS, CCRP Study Coordinator*
Phone: 317-274-7546
Email: wilmesk@iu.edu

**Sample Shipment Mailing address**

NCRAD
Indiana University School of Medicine
351 West 10th Street
TK-342
Indianapolis, IN 46202
3.2 Hours of Operation

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

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

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

3.3 Holiday Schedules

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

3.4 Holiday Observations

<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>3rd Monday in January</td>
<td>Martin Luther King, Jr Day</td>
</tr>
<tr>
<td>4th Monday in May</td>
<td>Memorial Day</td>
</tr>
<tr>
<td>July 4</td>
<td>Independence Day (observed)</td>
</tr>
<tr>
<td>1st Monday in September</td>
<td>Labor Day</td>
</tr>
<tr>
<td>4th Thursday in November</td>
<td>Thanksgiving</td>
</tr>
<tr>
<td>4th 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 24th and January 2nd, Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2nd. If at all possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University after the second week in December. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 20th by e-mailing alzstudy@iu.edu, so that they can arrange to have staff available to process incoming samples.

Please see: [https://ncrad.org/holiday_closures.html](https://ncrad.org/holiday_closures.html) for additional information.
4.0 NCRAD LABORATORY COLLECTION

4.1 Site Required Equipment

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

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

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

- Centrifuge capable of \( \geq 2000 \text{ rcf} \) with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:
Dry ice (about approximately 30-45 lbs per shipment)

4.2 BIOSPECIMENS COLLECTION SCHEDULES (SPECIMENS SENT TO NCRAD ONLY)

ADDS Blood Based Biomarker Collection Schedule for NCRAD:

<table>
<thead>
<tr>
<th></th>
<th>Baseline (1A)</th>
<th>3-Month (1B)</th>
<th>16-Month (2A)</th>
<th>19-Month (2B)</th>
<th>32-Month (3A)</th>
<th>35-Month (3B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

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

Consent forms must specify that any biological samples and de-identified clinical data may be shared with academic and/or industry collaborators through
NCRAD. A copy of the consent form for each subject should be kept on file by the site investigator.

Frozen samples are to be submitted according to the shipping methods outlined in Section 8.1. Guidelines for the processing, storage location, and timing of sample collection are listed in the tables below.
4.3 BIOSPECIMEN COLLECTION CHART

4.3.1 Blood Collection

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

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

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD; 2) CSF collection kits including Lumbar Puncture (LP) trays, the CSF Supplemental Supply Kit and the CSF Shipping Supply Kit; and 3) clinical lab supplies (with the exception of dry ice and equipment supplies listed in Section 4.1). These materials include blood tubes, pipettes, pipette tips, LP trays (when applicable), boxes for plasma/buffy coat/serum/CSF aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, Site and Subject ID Labels, Collection and Aliquot Tube Labels will all be provided by NCRAD. Details regarding the CSF Kits are found in the ABC-DS LP Manual of Procedures. Collection and Aliquot Tube Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 6.1.

5.1 **Specimen Collection Kit Contents**

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

### ADDS Blood Kit

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

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

### Blood Supplemental Supply Kit

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

### Individual Supplies

Available upon request within the NCRAD kit module.

<table>
<thead>
<tr>
<th>Quantities</th>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>By Request</td>
<td>25 cell cryobox</td>
</tr>
<tr>
<td>By Request</td>
<td>Siliconized Cryovial tube (0.5) with clear cap with lavender sticker</td>
</tr>
<tr>
<td>By Request</td>
<td>Siliconized Cryovial tube (0.5) with clear cap with red sticker</td>
</tr>
<tr>
<td>By Request</td>
<td>Cryovial tube (2 ml) with blue cap</td>
</tr>
<tr>
<td>By Request</td>
<td>FedEx return airbill</td>
</tr>
<tr>
<td>By Request</td>
<td>15 ml conical tube</td>
</tr>
<tr>
<td>By Request</td>
<td>Small IATA shipping box for ambient shipping</td>
</tr>
<tr>
<td>By Request</td>
<td>Shipping container for dry ice shipment (shipping and Styrofoam box)</td>
</tr>
<tr>
<td>By Request</td>
<td>Plastic biohazard bag</td>
</tr>
<tr>
<td>By Request</td>
<td>Disposable graduated transfer pipette</td>
</tr>
<tr>
<td>By Request</td>
<td>EDTA (Lavender-Top) Blood Collection Tube (10 ml)</td>
</tr>
<tr>
<td>By Request</td>
<td>Serum Separator (Gold-Top) Blood Collection Tube (5 ml)</td>
</tr>
</tbody>
</table>
5.2 Kit Supply to Study Sites

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

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

6.0 Blood Collection and Processing Procedures

***Important Note***

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

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

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

6.1 Labeling Samples

**Label Type Summary**

1. Kit Number Label
2. Collection and Aliquot Tube Label
3. Site and Subject ID Label
The **Kit Number Labels** do not indicate a specimen type, but are affixed on the Biological Sample and Shipment Notification Forms and on specific packing materials.

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

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

**Important Note**

Each collection tube will contain two labels: the Collection and Aliquot Tube Label and the Site and Subject ID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten Site and Subject ID label.
In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place blood collection and aliquot labels on ALL collection and aliquot tubes BEFORE sample collection, sample processing, or freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.

- Place cryovials in numerical order based on the specimen number, located at the top of the label. This ensures that no aliquot is misplaced or lost during the shipment process. (see Plasma Collection and Aliquot Tube Label on next page)
• Using a fine point permanent marker, fill-in and place the Site and Subject ID Labels on the collection tubes only (EDTA and SST) **BEFORE** sample collection, processing, or freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place Site and Subject ID labels on any cryovials.
• The Collection and Aliquot Tube Labels contain a 2D barcode on the left hand side of the label. Place this barcode toward the tube cap.
• Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see attached labeling diagram).
• Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label.

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

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

6.3 Filling Aliquot Tubes (Plasma and Serum)

In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each cryovial should be filled to 0.25 milliliters (see picture below) with the respective biological material after processing is completed (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample. If there is biologic material remaining that will not fill a subsequent cryovial, that remaining amount should still be included and shipped to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. You do not have to fill all cryovial tubes provided; you should attempt to fill as many tubes as possible with 0.25 ml of sample. For example, if 3.6 ml of sample is obtained, you should fill 14 cryovial tubes each with 0.25 ml, and one additional cryovial tube with the remaining 0.1 ml.
Please note: It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume (anything under 0.25 ml). Please record the specimen number and volume of the residual aliquot on the Biological Sample and Notification Form.

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

<table>
<thead>
<tr>
<th>Cap Color</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear Cap with Lavender Sticker</td>
<td>Plasma</td>
</tr>
<tr>
<td>Blue Cap</td>
<td>Buffy Coat</td>
</tr>
<tr>
<td>Clear Cap with Red Sticker</td>
<td>Serum</td>
</tr>
</tbody>
</table>

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

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

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

2. Place completed Site and ADDS ID Label and Collection and Aliquot “SERUM” Tube Labels on the Serum Separator (Gold-Top) Blood Collection Tubes (2 x 5 ml). Place pre-printed Collection and Aliquot “SERUM” Tube Labels on the (21) 0.5 ml siliconized cryovial tubes with clear caps and red stickers.
3. Using a blood collection set and a holder, collect blood into Serum Separator (Gold-Top) Blood Collection Tubes (2 x 5 ml) using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:
   a. Place donor’s arm in a downward position.
   b. Hold tube in a vertical position, below the donor’s arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. Ensure that the blood has stopped flowing into each tube before removing the tube from the holder. The tube with its vacuum is designed to draw 5 ml of blood into the tube.
   a. If complications arise during the blood draw, please note the difficulties on the ‘Biological Sample and Shipment Notification Form’. Do not attempt to draw an additional SST at this time. Process blood obtained in existing SST tube.

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

6. **CRITICAL STEP:** Allow blood to clot at room temperature by placing it upright in a vertical position in a tube rack for 30 minutes. Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.

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

   - Equivalent rpm for spin at 2000 x g
   - While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form Appendix B.
   - Serum samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
   - Record time aliquoted on the Biological Sample Shipment and Notification Form.
8. Remove the serum by tilting the tube and placing the pipette tip along the lower side of the wall. Transfer serum from both gold top Serum Separator tubes into the single 15ml conical tube. Mix the serum by gently inverting the conical tube 3-4 times.

9. Using a pipette, transfer serum from the 15 ml conical tube, into the pre-labeled siliconized cryovials with the red stickers on the caps. Aliquot 0.25 ml per cryovial (total vials=16-21 with 0.25 ml each). One Gold-Top tube should yield, on average, 5 ml of blood serum. Between both of the Gold-Top tubes, there should be an average of 10 ml of serum, for a total of 16-21 0.5 ml aliquot cryovial tubes per subject with 0.25 ml per cryovial tube. Be sure to only place serum in siliconized cryovials labeled with the “SERUM” label and red stickers on the caps. If there is extra serum left, use 1 extra cryovial provided for another <0.25 ml aliquot of serum and label as appropriate. If a residual aliquot (<0.25 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.
10. Place the labeled cryovials in the 25 cell cryobox and place on dry ice. Transfer to **-80°C Freezer when possible.** Store all samples at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

11. Dispose of collection tube with gel matrix and red blood cells at the bottom of the tube and empty 15ml conical tube according to your site’s guidelines for disposing of biomedical waste.
Immediately after blood draw, invert tube 5 times to mix samples.

- Store tubes at room temperature.
- Label tubes with preprinted labels prior to blood draw.
- Collect blood in (2) 5 mL Gold-Top tubes allowing blood to flow for 10 seconds and ensure blood flow has stopped.
- Immediately after blood draw, invert tube 5 times to mix samples.
- Allow blood to clot for 30 minutes.
- Within 60 minutes of blood draw, centrifuge samples at 2000 x g at 4°C for 10 minutes
- Using a clean transfer pipette, transfer Serum from both 5 mL Gold-Top tubes to the 15 mL conical tube.
- Mix the 15 mL conical tube gently by inverting 3-4 times.
- Adhere preprinted labels to the clear cap cryovials with red stickers.
- Aliquot 0.25 ml into each cryovial tube.
- If a residual aliquot is created, document specimen number on Sample Notification Form.
- Store serum aliquots at -80°C until shipment.
6.5 EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Plasma and Buffy Coat

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

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

2. Place completed Site and Subject ID Label and pre-printed “PLASMA” Collection and Aliquot Tube Label on the lavender-top EDTA tube. Place pre-printed “PLASMA” Collection and Aliquot Tube Labels on the (21) 0.5 ml siliconized cryovial tubes with clear caps and lavender stickers. Place pre-printed “BUFFY COAT” Collection and Aliquot Tube Label on the (1) 2 ml cryovial with a blue lid.

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

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

   The following techniques shall be used to prevent possible backflow:

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

5. Allow at least 10 seconds for a complete blood draw to take place in each tube. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder. The tube with its vacuum is designed to draw 10 ml of blood into the tube.
   a. If complications arise during the blood draw, please note the difficulties on the ‘Biological Sample and Shipment Notification Form’. Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.

6. CRITICAL STEP: Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 5 times.
7. **CRITICAL STEP:** Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.

- Preferably within 30 minutes of blood collection, centrifuge balanced tubes for 10 minutes at 2000 RCF (x g) at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM).**
- Equivalent rpm for spin at 2000 x g
- While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
- Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
- Record time aliquoted on the Biological Sample and Shipment Notification Form.

8. Remove the plasma, being careful not to agitate the packed red blood cells at the bottom of the tube. Tilt the tube and placing the disposable pipette tip along the lower side of the wall without touching the pellet (buffy coat) so that plasma is not contaminated (see below). Transfer plasma into the pre-labeled cryovials. Aliquot 0.25 ml per cryovial (total vials = 16-21 with 0.25 ml each). The EDTA tube should yield, on average, 5 ml of plasma for a total of 16-21 0.5 ml siliconized cryovial tubes per subject with 0.25 ml per cryovial tube. Be sure to only place plasma in cryovials labeled with “PLASMA” labels. Take caution not to disturb the red blood cells at the bottom of the tube. If there is extra plasma left, use 1 extra cryovial provided for another <0.25 ml aliquot of plasma. **If a residual aliquot (<0.25 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**
9. Place the labeled cryovials in the 25 cryovial box and place on dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.
10. After plasma has been removed from the EDTA (Lavender-Top) Blood Collection Tube (10 ml), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs—see figure) into labeled cryovial with blue cap using a disposable graduated micropipette. All of the buffy coat will be placed into one cryovial. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovial with the blue cap and “BUFFY COAT” label.

11. Dispose of tube with red blood cell pellet according to your site’s guidelines for disposing of biomedical waste.

12. Place the labeled cryovial in the 25 cryovial box and place on dry ice. Transfer to -80°C Freezer when possible. Store all samples at -80°C until shipped to NCRAD on dry ice.
Plasma and Buffy Coat Preparation (10ml Lavender-Top Tube)

**Step One**
- Store tubes at room temperature.
- Label tubes with preprinted labels prior to blood draw.

**Step Two**
- Collect blood in Plasma Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

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

**Step Four**
- Place thoroughly mixed tube on wet ice until centrifugation begins.
- Preferably within 30 minutes of blood draw, centrifuge samples at 2000 x g at 4°C for 10 minutes.
- Samples need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.

**Step Five**
- Plasma

**Step Six**
- Adhere preprinted labels to the clear cap cryovials with lavender stickers.
- Aliquot 0.25 ml into each cryovial tube.
- If a residual aliquot is created, document specimen number and volume on Sample Notification Form.
- Store plasma aliquots at -80°C until shipment.

**Step Seven**
- Adhere preprinted labels to the blue cap cryovial.
- Using a clean pipette tip, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat into the cryovial tube.
- Store buffy coat aliquot at -80°C until shipment.
7.0 **INCOMPLETE OR DIFFICULT BLOOD DRAWS**

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

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

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

1. **If the biospecimens at a scheduled visit are partially collected:**
   a. Attempt to process and submit any samples that were able to be collected during the visit
   b. Document difficulties on the ‘Biological Sample and Shipment Notification Form’ prior to submission to NCRAD
      i. Indicate blood draw difficulties at the bottom of the ‘Biological Sample and Shipment Notification Form’ within the “Notes” section.
      ii. Complete the 'Biological Sample and Shipment Notification Form' with tube volume approximations and number of aliquots created.
   c. Contact a NCRAD coordinator and alert them of the challenging blood draw

2. **If the biospecimens at a scheduled visit are not collected:**
   a. Contact the ADDS Global Coordinator and a NCRAD coordinator to alert them of the challenging blood draw or circumstances as to why biospecimens were not collected.
   b. Schedule participant for a longitudinal visit.

8.0 **PACKAGING AND SHIPPING INSTRUCTIONS**

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

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

**IMPORTANT!**

FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY!

Specimens being shipped to NCRAD should be considered as Category B UN3373 specimens and as such must be tripled packaged and compliant with IATA Packing Instructions 650. See the Latest Edition of the IATA Regulations for complete documentation.
Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.
8.1 Frozen Shipment Instructions

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

2. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu

Attach the following to the email:

- Completed Biological Sample and Shipment Notification Form to the email notification. (See Appendix B for an example of the NCRAD sample form)

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

3. Place all frozen labeled aliquots of plasma and buffy coat in the cryovial cryobox.

   i. Each cryobox holds 25 samples and there will be approximately 40 cryovial samples. Please place plasma and buffy coat aliquots within one cryobox and the serum aliquots within the second cryobox. (21 plasma, 21 serum, and 1 buffy coat) per blood draw (see below).

   ii. Cryoboxes should contain all of the specimens from the same patient, per time point.

   iii. Batch shipping should be performed every 3 months or when specimens from 5 participants accumulates, whichever is sooner.
4. Label the outside of the cryoboxes with the kit number label. Place Plasma/Buffy Coat aliquots within one cryobox and the serum aliquots in another cryobox. BOTH cryoboxes should be placed within the SAME biohazard bag. These biohazard bags are large enough to contain one or two cryoboxes.

5. Place the cryoboxes in the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag.

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

7. Place the biohazard bag into the provided Styrofoam-lined shipping container on top of the dry ice. Please ensure that cryoboxes are placed so the cryovials are upright in the shipping container. Layer dry ice and cryoboxes as necessary.

8. The inner Styrofoam shipping container must contain approximately 30-45 lbs (or ~21kg) of dry ice. The dry ice should entirely fill the inner box to ensure the frozen state of the specimens.

Full Shipping Container with Batched Samples and Dry Ice
9. Replace the lid on the Styrofoam carton. Place the completed Biological Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape.

10. Complete the FedEx return airbill with the following information:
   a. Section 1, “From”: fill in your name, address, phone number, and Site FedEx Account Number.
   b. Section 2, “Your Internal Billing Reference”: add any additional information required by your site.
   c. Section 6, “Special Handling and Delivery Signature Options”: under “Does this shipment contain dangerous goods?” check the boxes for “Yes, Shipper’s Declaration not required” and “Dry Ice”. Enter the number of packages (1) x the net weight of dry ice in kg.
   d. Section 7, “Payment”, check third party and bill transportation costs to the ADDS study FedEx account number.

11. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
   a. Your name and return address
   b. Net weight of dry ice in kg (must match amount on the airbill)
   c. Consignee name and address:
      NCRAD
      IU School of Medicine
      351 West 10th Street
      TK-342
      Indianapolis, IN 46202
   d. Do not cover any part of this label with other stickers, including pre-printed address labels.

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

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

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

NCRAD
Walther Hall – R3-C102
351 West 10th Street
TK-342
Phone: 1-800-526-2839

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

***Important Note***
For frozen shipments, include no more than ten cryovial boxes (separated by patient within 5 biohazard bags) per shipping container in order to have room for a sufficient amount of dry ice to keep samples frozen up to 24 hours.
The labeled, processed, aliquoted, and frozen cryovials of serum, plasma and buffy coat will be shipped to NCRAD as outlined above.

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

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

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

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

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

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

- Missing samples
- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Biological Sample and Shipment Notification Form and logged at NCRAD compared to information entered into the DCC database.
- Samples that are frozen and stored longer than one quarter at the site
- Use of an incorrect Biological or CSF Sample and Shipment Notification Form

10.0 **APPENDICES LIST**

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

[Appendix B: Biological Sample and Shipment Notification Form](#)
Appendix A
Rate of Centrifuge Worksheet

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

Submitter Information
Name: 
Submitter e-mail: 
Site: 

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

317-278-1100 (Fax) alzstudy@iu.edu
### Appendix B

**Biological Sample and Shipment Notification Form**

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

<table>
<thead>
<tr>
<th>General Information:</th>
</tr>
</thead>
<tbody>
<tr>
<td>To: Kelley Faber Email: <a href="mailto:alzstudy@iu.edu">alzstudy@iu.edu</a></td>
</tr>
<tr>
<td>FAX: 317-278-1100 Phone: 1-800-526-2839</td>
</tr>
<tr>
<td>Date: ___________________________</td>
</tr>
<tr>
<td>Email: ___________________________</td>
</tr>
</tbody>
</table>

#### Blood Collection:

1. **Date Drawn:** ___________ [YYYYMMDD]
2. **Time of Draw (24 hour clock):** ___________ [HHMM]
3. **Last time subject ate (Date):** ___________ [YYYYMMDD]
4. **Last time subject ate (24 hour clock):** ___________ [HHMM]
5. **Was the EDTA tube placed on ice immediately after inverting tube 5 times until centrifugation began?** 
   - Yes
   - No

#### Blood Processing:

**Plasma (EDTA/Lavender 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 (1x10 mL EDTA tube):** ___________ mL
- **Time aliquoted:** ___________ [HHMM]

**Number of 0.25 mL plasma aliquots created (16-20 total)**

(Siliconized cryovial):

- ___________ x 0.25 mL

**Serum (Serum Separator/Gold Top Tube)**

- **Time spin started (24 hour clock) (30 minutes after draw time):** ___________ [HHMM]
- **Duration of centrifuge:** ___________ [minutes]
- **Temp of centrifuge:** ________ °C • Rate of centrifuge: ________ x g
- **Original volume drawn (2x6 mL Serum tube):** ___________ mL
- **Time aliquoted:** ___________ [HHMM]

**Number of 0.25 mL serum aliquots created (16-20 total)**

(Siliconized cryovial):

- ___________ x 0.25 mL

- **If applicable, volume of residual plasma aliquot (less than 0.25 mL)** (Siliconized cryovial):
  - ___________ mL

- **If applicable, specimen number of residual aliquot** (Last four digits):

- **Time aliquots placed in freezer (24 hour clock):** ___________ [HHMM]
- **Storage temperature of freezer:** ________ °C

- **Buffy coat aliquot created (one per EDTA tube) (Blue cap cryovial):** ___________ mL

*Note: Bulleted items not entered into eCRF.

**Version 07.2018**