**LIFE-DSR MANUAL OF PROCEDURES UPDATE:**

V07.2021

<table>
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<th>Section</th>
<th>Change</th>
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<tr>
<td>Document Footer</td>
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<tr>
<td>Title Page</td>
<td>Title page updated to reflect version change.</td>
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<tr>
<td>Throughout document</td>
<td>1. Updated to reflect addition of 10ml EDTA tubes</td>
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<td>Section 8.2</td>
<td>1. Updated UPS Shipping information.</td>
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Longitudinal Investigation for Enhancing Down Syndrome Research (LIFE-DSR) Study
of the
Down Syndrome – Clinical Trials Network
in collaboration with
The National Centralized Repository for Alzheimer’s Disease and Related Dementias (NCRAD)

Biofluid Collection, Processing and Shipment Manual of Procedures
Version 5.0
July, 2021
# TABLE OF CONTENTS

1.0  **Abbreviations** ........................................................................................................... 4

2.0  **Purpose** ...................................................................................................................... 5

3.0  **NCRAD Information** ................................................................................................. 6
   3.1  **NCRAD Contacts**
   3.2  **Hours of Operation**
   3.3  **Holiday Schedules**
   3.4  **Holiday Observations**

4.0  **NCRAD Laboratory Collection** ................................................................................. 8
   4.1  **Site Required Equipment**
   4.2  **Biofluid Collection Schedules**
   4.3  **Biofluid Collection Charts**

5.0  **Specimen Collection Kits, Shipping Kits, and Supplies** ............................................... 9
   5.1  **Specimen Collection Kit Contents**
   5.2  **Kit Supply to Study Sites**

6.0  **Blood Collection and Processing Procedures** ............................................................ 11
   6.1  **Labeling Samples**
   6.2  **Video List**
   6.3  **Filling Aliquot Tubes (Plasma)**
   6.4  **EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Plasma and Buffy Coat**

7.0  **Incomplete or Difficult Blood Draws** ........................................................................ 23

8.0  **Packaging and Shipping Instructions** ........................................................................ 23
   8.1  **Frozen Packaging Instructions**
   8.2  **Frozen Shipping Instructions**

9.0  **Data Queries and Reconciliation** .............................................................................. 29

10.0 **Appendices List** ....................................................................................................... 31
    **Appendix A: Rate of Centrifuge Worksheet**
    **Appendix B: Biological Sample and Shipment Notification Form**
## 1.0 Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DS-CTN</td>
<td>Down Syndrome Clinical Trials Network</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>LIFE-DSR</td>
<td>Longitudinal Investigation for Enhancing Down Syndrome Research</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>UPS</td>
<td>United Parcel Service</td>
</tr>
</tbody>
</table>
2.0 PURPOSE

The collection of biofluids is an important part of the Longitudinal Investigation for Enhancing Down Syndrome Research (LIFE-DSR) Study of the Down Syndrome – Clinical Trials Network (DS-CTN). 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 biological samples for LIFE-DSR 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)

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 specimens being provided to NCRAD for the LIFE-DSR protocol.
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

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

**General NCRAD Contact Information**
Phone: 1-800-526-2839
Fax: 317-321-2003
Email: alzstudy@iu.edu
Website: [www.ncrad.org](http://www.ncrad.org)
LIFE-DSR Study Specific Webpage: [https://ncrad.org/resource_life-dsr.html](https://ncrad.org/resource_life-dsr.html)

**Sample Shipment Mailing Address**
LIFE-DSR at NCRAD
Indiana University School of Medicine
351 West 10th Street
TK-217
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 of 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.

- 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.
- **Although rarely occurs, weekend/holiday delivery must be arranged in advance with NCRAD staff.**
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 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 $\geq 2000 \times g$ 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 **BIOFLUID COLLECTION SCHEDULES**

**LIFE-DSR Collection Schedule:**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>16M</th>
<th>32M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DNA</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Whole blood is collected in one type of tube (10ml lavender-top EDTA tube). The 10ml EDTA tubes are processed locally into plasma and buffy coat fractions; 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 participant 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 BIOFLUID COLLECTION CHARTS

4.3.1 Biofluid Collection for Baseline, 16M and 32M Visits

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Tube Type</th>
<th>Number of Tubes Supplied in Kit</th>
<th>Aliquot Volume</th>
<th>Tubes to NCRAD</th>
<th>Ship</th>
</tr>
</thead>
<tbody>
<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>5</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>PLASMA: 2.0 ml cryovials with lavender cap (residual volume placed in 2.0 ml cryovial with blue cap)</td>
<td>17</td>
<td>1.5 ml plasma aliquot per 2.0 ml cryovial (lavender cap)</td>
<td>Up to 17</td>
<td>Frozen</td>
</tr>
<tr>
<td></td>
<td>BUFFY COAT: 2.0 ml cryovial</td>
<td>5</td>
<td>1 ml buffy coat aliquot per 2.0 ml cryovial (clear cap)</td>
<td>5</td>
<td>Frozen</td>
</tr>
</tbody>
</table>

If a sample is not obtained at a particular visit, it 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 and track it as a protocol deviation.

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, the Blood Supplemental Supply Kit, the Frozen Shipment Supply Kit 2) clinical lab supplies (with the exception of dry ice and equipment supplies listed in Section 4.1). The provided materials include blood tubes, pipettes, boxes for plasma/buffy coat aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, LIFE-DSR ID Labels, Collection and Aliquot Tube Labels will all be provided by NCRAD. Details regarding the blood kits are found in this Manual of Procedures. Collection and Aliquot Tube Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 6.1.
5.1 Specimen Collection Kit Contents

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

**LIFE-DSR Blood-Based Kits**

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Blood-Based Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>EDTA (Lavender-Top) Blood Collection Tube (10 ml)</td>
</tr>
<tr>
<td>16</td>
<td>Cryovial tube (2.0 ml) with lavender cap</td>
</tr>
<tr>
<td>1</td>
<td>Cryovial tube (2.0 ml) with blue cap</td>
</tr>
<tr>
<td>5</td>
<td>Cryovial tube (2.0 ml) with clear cap</td>
</tr>
<tr>
<td>2</td>
<td>Disposable graduated transfer pipette</td>
</tr>
<tr>
<td>1</td>
<td>50ml conical</td>
</tr>
<tr>
<td>27</td>
<td>Pre-printed Collection and Aliquot Tube Label</td>
</tr>
<tr>
<td>3</td>
<td>Pre-printed Kit Number Label</td>
</tr>
<tr>
<td>6</td>
<td>Labels for handwritten LIFE-DSR ID</td>
</tr>
<tr>
<td>1</td>
<td>Cryovial box (holds up to 25 cryovials)</td>
</tr>
<tr>
<td>1</td>
<td>Resealable bag</td>
</tr>
<tr>
<td>5</td>
<td>Resealable bubble wrap tube pouches</td>
</tr>
</tbody>
</table>

**Blood-Based Supplemental Supply Kit**

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Blood-Based Supplemental Supply Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>EDTA (Lavender-Top) Blood Collection Tube (10 ml)</td>
</tr>
<tr>
<td>25</td>
<td>Cryovial tube (2.0 ml) with lavender cap</td>
</tr>
<tr>
<td>5</td>
<td>Cryovial tube (2.0 ml) with blue cap</td>
</tr>
<tr>
<td>15</td>
<td>Cryovial tube (2.0 ml) with clear cap</td>
</tr>
<tr>
<td>5</td>
<td>Disposable graduated transfer pipette</td>
</tr>
<tr>
<td>5</td>
<td>50ml conical</td>
</tr>
<tr>
<td>10</td>
<td>Labels for handwritten LIFE-DSR ID</td>
</tr>
<tr>
<td>5</td>
<td>Cryovial box (holds up to 25 cryovials)</td>
</tr>
</tbody>
</table>

**LIFE-DSR Frozen Blood Shipping Supply Kit**

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Frozen Shipping Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Plastic Biohazard bag with absorbent sheet (small)</td>
</tr>
<tr>
<td>1</td>
<td>UPS return label</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>
### Individual Supplies

<table>
<thead>
<tr>
<th>Quantities</th>
<th>Items Available upon request within the NCRAD kit module.</th>
</tr>
</thead>
<tbody>
<tr>
<td>By Request</td>
<td>Cryovial box (holds up to 25 cryovials)</td>
</tr>
<tr>
<td>By Request</td>
<td>Cryovial tube (2.0 ml) with lavender cap</td>
</tr>
<tr>
<td>By Request</td>
<td>Cryovial tube (2.0 ml) with clear cap</td>
</tr>
<tr>
<td>By Request</td>
<td>Cryovial tube (2.0 ml) with blue cap</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>Styrofoam shipping containers (11”x9”x8” 1 1/2” wall)</td>
</tr>
<tr>
<td>By Request</td>
<td>Plastic biohazard bag with absorbent sheet (small)</td>
</tr>
<tr>
<td>By Request</td>
<td>Disposable graduated transfer pipette</td>
</tr>
<tr>
<td>By Request</td>
<td>50ml conical</td>
</tr>
<tr>
<td>By Request</td>
<td>EDTA (Lavender-Top) Blood Collection Tube (10 ml)</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>UPS Dry ice shipping label</td>
</tr>
<tr>
<td>By Request</td>
<td>Fine point permanent markers</td>
</tr>
<tr>
<td>By Request</td>
<td>LIFE-DSR 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: [http://kits.iu.edu/lifedsr](http://kits.iu.edu/lifedsr) 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. **Please note that the centrifuge may take 30 minutes to cool, so please plan accordingly.**
6.1 Labeling Samples

**Label Type Summary**
1. Kit Number Label
2. Collection and Aliquot Tube Label
3. LIFE-DSR ID Label

The Kit Number Labels do not indicate a specimen type, but are affixed on the Biological Sample and Shipment Notification Form and on specific packing materials. This label ties together all specimens collected from one subject at one visit.

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

The LIFE-DSR ID Labels are placed on all collection tubes. This label is used to document the individual’s unique LIFE-DSR ID.
**Important Note**

Each collection tube will contain two labels: the Collection and Aliquot Tube Label and the LIFE-DSR 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 LIFE-DSR ID label below.

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.
- Using a fine point permanent marker, fill-in the LIFE-DSR ID Labels and place on the collection tubes only (EDTA) BEFORE sample collection, processing, or
freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place LIFE-DSR 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 labeling diagram below).
- 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.

![Aliquot Tube Labeling Diagram](image)

- 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_life-dsr.html](https://ncrad.org/resource_life-dsr.html)
    - LIFE-DSR MOP Training
    - Plasma and Buffy Coat Processing and Aliquoting
    - Frozen Shipping

### 6.3 Filling Aliquot Tubes (Plasma)
In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each cryovial should be filled to the assigned volume with the respective biological material after processing is completed (refer to detailed processing instructions for average yield per sample).

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

Aliquot the remaining biological material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 3.6 ml of sample is obtained, you should fill 2 cryovial tubes each with 1.5 ml, and one additional cryovial tube with the remaining 0.6 ml.

**Note:** It is critical for the integrity of the samples that study staff note if an aliquot tube contains a residual volume (anything under 1.5 ml). Please highlight that the aliquot contains a small volume by utilizing the blue cryovial cap provided in each kit. Please record the specimen number and volume of the residual aliquot on the Biological Sample and Notification Form.

To assist in the preparation and aliquoting of samples, colored caps 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>Lavender Cap</td>
<td>Plasma</td>
</tr>
<tr>
<td>Clear Cap</td>
<td>Buffy Coat</td>
</tr>
<tr>
<td>Blue Cap</td>
<td>Residual (plasma)</td>
</tr>
</tbody>
</table>
6.4 EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Plasma and Buffy Coat

Whole Blood Collection for Isolation of Plasma and Buffy Coat: 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 LIFE-DSR ID Label and pre-printed “PLASMA” Collection and Aliquot Tube Label on the lavender-top EDTA tubes. Place pre-printed “PLASMA” Collection and Aliquot Tube Labels on the (16) 2.0 ml cryovial tubes with lavender caps and (1) 2.0 ml cryovial tube with blue cap (if necessary, for residual). Place pre-printed “BUFFY COAT” Collection and Aliquot Tube Label on the (5) 2.0 ml cryovial with a clear lid.

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

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 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.

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 tubes 8-10 times.

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

8. Centrifuge balanced tubes for 10 minutes at 2000 x g and 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 1 hour from the time of collection.
      Record time aliquoted on the Biological Sample Shipment and Notification Form.

9. Remove the plasma, being careful not to agitate the packed red blood cells at the bottom of the tube. Tilt the tube and place a disposable pipette tip along the lower side of the wall without touching the pellet (buffy coat) so that plasma is not contaminated (see below). Transfer plasma from all five EDTA tubes into the 50 ml conical tube and gently invert 3 times. Aliquot 1.5 ml per cryovial (total vials = up to 17 with 1.5 ml each). Each EDTA tube should yield, on average, 4-5 ml of plasma. Be sure to only place plasma in cryovials with lavender caps and 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 <1.5 ml aliquot of plasma. **If a residual aliquot (<1.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**
NOTE: When pipetting plasma from the plasma tube into the cryovials, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.
10. 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 Shipment and Notification Form.

11. After plasma has been removed from the EDTA (Lavender-Top) Blood Collection Tubes (10 ml), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) into labeled cryovials with clear caps using a micropipette. Aliquot each buffy coat into a separate cryovial. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovials with the clear caps and “BUFFY COAT” label.
12. Dispose of tube with red blood cell pellet according to your site’s guidelines for disposing of biomedical waste.

13. Place the labeled cryovials in the 25 cryovial box and place on dry ice. Transfer to \(-80{\degree}\text{C Freezer when possible.}\) Store all samples at \(-80{\degree}\text{C until shipped}\) to NCRAD on dry ice.
Plasma and Buffy Coat Preparation (10ml Lavender-Top Tube x 5)

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

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

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

**Step Four**
- Place thoroughly mixed tubes on wet ice until centrifugation begins.

**Step Five**
- Centrifuge samples at 2000 x g at 4°C for 10 minutes.
- Samples need to be spun, aliquoted, and in the freezer within 1 hour from the time of collection.

**Step Six**
- Pool all plasma from the 5 EDTA tubes into a 50ml conical tube and invert gently 3 times to mix the plasma.

**Step Seven**
- Adhere preprinted labels to the lavender cap cryovials.
- Aliquot 1.5 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 Eight**
- Adhere preprinted labels to the clear cap cryovials.
- Using a clean pipette tip, collect the Buffy coats (may have residual plasma and some RBCs included).
- Transfer the Buffy coats into the cryovial tubes.
- Store Buffy coat aliquots 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.

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

1. If the biofluids 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 biofluids at a scheduled visit are not collected:
   a. Enter a protocol deviation into TrialMaster OmniComm, with a brief note about mitigating circumstances/reason/etc.
   b. Email the ADCS LIFE-DSR Study Team email (life-dsr@ucsd.edu) to alert them of the challenging blood draw or circumstances as to why biofluids were not collected.

8.0 **PACKAGING AND SHIPPING INSTRUCTIONS**

**ALL** study personnel responsible for shipping should be certified in biofluid shipping (i.e. IATA certification). If not available at your institution, please contact NCRAD with questions and information regarding resources.
<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Processing/ Aliquoting</th>
<th>Tubes to NCRAD</th>
<th>Ship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood (Lavender-Top EDTA) for isolation of plasma &amp; buffy coat (for DNA extraction)</td>
<td>1.5 ml plasma aliquots per 2.0 ml cryovial (lavender cap); residual volume placed in 2.0 ml cryovial with blue cap</td>
<td>Up to 17</td>
<td>Frozen</td>
</tr>
<tr>
<td></td>
<td>1 ml buffy coat aliquot per 2.0 ml cryovial (clear cap)</td>
<td>5</td>
<td>Frozen</td>
</tr>
</tbody>
</table>

### 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!**

FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY!
Specimens being shipped to NCRAD should be considered as Category B UN3373 specimens and as such must be tripled packaged and compliant with IATA Packing Instructions 650. See the Latest Edition of the IATA Regulations for complete documentation.

*** Packing and Labeling Guidelines ***

- The primary receptacle (frozen cryovials) must be leak proof and must not contain more than 1L total.
- The secondary packaging (biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle 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

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.

Frozen Packaging Instructions

1. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu
   Attach the following to the email:
   a. Completed Biological Sample and Shipment Notification Form to the email notification.
b. If email is unavailable, please call NCRAD (800-526-2839) and do not ship until you have contacted and notified NCRAD coordinators about the shipment in advance.

2. Place all frozen labeled aliquots of plasma and buffy coat aliquots from the same subject in the cryovial cryobox.
   a. Each 25-slot cryobox will hold approximately 22 cryovial samples. Place plasma and buffy coat within one cryobox (17 plasma, 5 buffy coat) per participant blood draw (see below).
   
   | One cryobox containing plasma, residual, and buffy coat aliquots. |
   
   b. Cryoboxes should contain all of the specimens from the same patient, per time point.
   c. **Batch shipping should be performed every (3) three months or when specimens from 5 participants accumulates, whichever is sooner.**

3. Label the outside of the cryoboxes with the appropriate kit number label(s). Place plasma and buffy coat aliquots within one cryobox and place within a biohazard bag. The biohazard bags are large enough to contain one cryobox from one subject’s visit.

4. Place the cryobox in the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag). Seal biohazard bag according to the instructions on the bag.
5. Place approximately 2-3 inches of dry ice in the bottom of the Styrofoam shipping container.
6. 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 (as pictured below).
7. Fully cover the cryoboxes and tubes with approximately 2 inches of dry ice.
8. The inner Styrofoam shipping container must contain approximately 45 lbs (or 21kg) of dry ice. The dry ice should entirely fill the inner box to ensure the frozen state of the specimens.

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.

a. Attach provided UPS label for packages.

10. Complete the UPS Dry Ice Label
   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 pre-printed address labels.

11. Apply all provided warning labels and the pre-printed UPS return airbill to the outside of package, taking care not to overlap labels.

       IMPORTANT!
       Ensure UPS address label is attached and UPS Dry Ice label is filled out, or UPS may reject or return your package.

12. Hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off.
13. 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. UPS does not replenish dry ice if shipments are delayed or held over during the weekend.

       LIFE-DSR at NCRAD
       Indiana University School of Medicine
       351 West 10th Street
       TK-217
       Indianapolis, IN 46202
       Phone: 1-800-526-2839

14. 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.

       ***Important Note***
       For frozen shipments, include no more than five 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 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 INCLEMENT WEATHER THAT MAY DELAY SHIPMENT/DELIVERY OF SAMPLES
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 your 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.

Remember to complete the Biological Sample and Shipment Notification Forms Appendix B - include a copy in your shipment AND notify the NCRAD Study Coordinator by email at alzstudy@iu.edu (include UPS tracking number in email) IN ADVANCE to confirm the shipment.
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. Ambient shipments
      i. Enter the total weight of your package in the “Weight” field and leave the “Dry Ice Weight” field empty.
   b. 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.
   c. Click Ship in the bottom right of the page when complete.

7. If your site does not already have a daily UPS pickup, you will need to schedule one
   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.
   b. Click Detailed Report from the dropdown menu on the right side of the page.
   c. Enter tracking number if known. Otherwise, search by ship date. Click Search.
   d. 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.

11. A UPS Pickup is automatically scheduled at the address you are shipping from, and the pickup is charged to NCRAD.
   a. If shipment occurs too late in the day for an automatic UPS pickup, you will receive an email stating that the pickup could not be scheduled, and you will need to make other arrangements.

9.0 **DATA QUERIES AND SAMPLE RECONCILIATION**

The Laboratory worksheets must be completed on the day that samples are collected since they capture information related to the details of the sample collection and processing. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.
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 ADCS database.
- Samples that are frozen and stored longer than one quarter at the site
- Use of an incorrect Biological 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:

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

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

Comments:

Please send this form to NCRAD Study Coordinator

317-321-2003 (Fax)  
alzstudy@iu.edu
## Appendix B

### Biological Sample and Shipment Notification Form

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

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

### General Information:
- **Participant ID:** DSR — — — — — — — — — —
- **UPS tracking #:** ___________
- **From:** ___________  
- **Date:** ___________
- **Phone:** ___________  
- **Email:** ___________

### Study: LIFE-DSR
- **Visit (circle one):** BASELINE  
- **Month 16**  
- **Month 32**
- **Kit #:**  
- **KIT BARCODE**
- **Sex:**  
  - [ ] M  
  - [ ] F  
  - **Year of Birth:** ___________

### Blood Collection:
1. **Date Drawn:** [MMDDYY]  
2. **Time of Draw:** [HHMM]
3. **Last time subject ate:** [MMDDYY]  
4. **Last time subject ate:** [HHMM]

### Blood Processing:

#### Plasma & Buffy Coat (Lavender-top) Tube (10 mL)
- **Time spin started:** [HHMM]
- **Duration of centrifuge:** ________ Minutes
- **Temp of Centrifuge:** ________ °C  
  - **Rate of centrifuge:** ________ x g
- **Time aliquoted:** [HHMM]

#### Number of 1.5 mL plasma aliquots created (lavender cap):
- ___________

#### If applicable, volume of residual plasma aliquot (less than 1.5 mL in blue cap):
- ___________ mL

#### If applicable, specimen number of residual plasma aliquot (last four digits):
- ___________

#### Buffy coat #1 last four digits of specimen number:
- ___________

#### Buffy coat #1 volume:
- ___________ mL  
  - **Original blood volume drawn:** ___________ mL

#### Buffy coat #2 last four digits of specimen number:
- ___________

#### Buffy coat #2 volume:
- ___________ mL  
  - **Original blood volume drawn:** ___________ mL

#### Buffy coat #3 last four digits of specimen number:
- ___________

#### Buffy coat #3 volume:
- ___________ mL  
  - **Original blood volume drawn:** ___________ mL

#### Buffy coat #4 last four digits of specimen number:
- ___________

#### Buffy coat #4 volume:
- ___________ mL  
  - **Original blood volume drawn:** ___________ mL

#### Buffy coat #5 last four digits of specimen number:
- ___________

#### Buffy coat #5 volume:
- ___________ mL  
  - **Original blood volume drawn:** ___________ mL

#### Time aliquots placed in freezer:
- ___________ [HHMM]

#### Storage temperature in freezer:
- ___________ °C

### Notes:

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**Version (07.2021)**