# ABC-DS Manual of Procedures Update

## V09.2022

<table>
<thead>
<tr>
<th>Section</th>
<th>Change</th>
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<tbody>
<tr>
<td>Document Footer</td>
<td>The version date was updated for this amendment.</td>
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<tr>
<td>Title Page</td>
<td>Title page updated to reflect version change.</td>
</tr>
</tbody>
</table>
2. Removal of Serum Discard Tube due to recent confirmation that Serum Discard Tube is not required to be drawn prior to drawing RNA PAXGene™ tube unless the RNA PAXGene™ is the only tube drawn. |
Alzheimer’s Biomarker Consortium – Down Syndrome

in collaboration with

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

Blood-Based Biospecimens

Manual of Procedures

Version 2.3

September 2022
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# 1.0 Abbreviations

<table>
<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>APOE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>ATRI</td>
<td>Alzheimer’s Therapeutic Research Institute</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>IU Path Lab</td>
<td>Indiana University Pathology Lab</td>
</tr>
<tr>
<td>LP</td>
<td>Lumbar Puncture</td>
</tr>
<tr>
<td>miRNA</td>
<td>Micro Ribonucleic Acid</td>
</tr>
<tr>
<td>NaHep</td>
<td>Sodium Heparin (Green-Top) Blood Collection Tube (4 mL)</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>RNA</td>
<td>Ribonucleic Acid</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>
<tr>
<td>UNTHSC</td>
<td>University of North Texas – Health Science Center</td>
</tr>
</tbody>
</table>
2.0 PURPOSE

The collection of blood-based biofluids is an important part of the Alzheimer’s Biomarker Consortium – Down Syndrome (ABC-DS) study. These samples will be used to:

- Identify critical factors important to neurodegeneration and, ultimately, dementia
- Define biomarkers for the above factors
- 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 for ABC-DS study visits.

**The following samples will be sent to NCRAD:**

- Serum
- Sodium Heparin (NaHep) – (karyotyping)
- Plasma
- Buffy Coat – (DNA extraction)
- RNA – (miRNA extraction)

**Additional samples collected but not shipped to NCRAD:**

- Serum – shipped to UNTHSC
- Plasma – shipped to UNTHSC
- CSF – shipped to Washington University – St. Louis (Please see the ABC-DS Lumbar Puncture Manual of Procedures for details)
- Clinical Labs – shipped to IU Path Lab
  - Serum 5.0mL orange-top x1
  - Serum 5.0mL gold-top x1
  - EDTA 3.0mL x1

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

These procedures are relevant to all study personnel responsible for processing blood specimens entering UNTHSC, NCRAD and IU Path Lab.
3.0 CONTACT INFORMATION

3.1 NCRAD Contacts

Tatiana Foroud, PhD, NCRAD Principal Investigator
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

Zoë Potter, BA, Study Coordinator
Phone: (317) 278-9086
Email: zdpotter@iu.edu

General NCRAD Contact Information
Phone: 1-800-526-2839
Email: alzstudy@iu.edu
Website: www.ncrad.org
ABC-DS Study Specific Webpage:
https://www.ncrad.org/resource_ABC-DS.html

3.2 Sample Shipment Mailing Addresses:

**NCRAD**
Indiana University School of Medicine
351 West 10th Street
TK-217
Indianapolis, IN 46202
alzstudy@iu.edu

**UNTHSC**
3420 Darcy Street
Fort Worth, TX 76107
Phone: 817-735-2638
Tori.Como@unthsc.edu

**IU Path Lab**
350 W. 11th Street
5th Floor, Rm 5013
Indianapolis, IN 46202
Karen Cleary: kcleary@IUHealth.org
Patti Jordan: Pjordan@IUHealth.org
Rustin Ball: rball3@IUHEALTH.ORG
Jessica Minch: jminch1@iuhealth.org
3.3 Hours of Operation

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

**UNTHSC** business hours are from 8 AM to 5 PM *Central 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.

3.4 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>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>June 19</td>
<td>Juneteenth (observed)</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 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.
- For holiday observations regarding shipments to UNTHSC, please follow the guidelines as outlined above for Indiana University.
- 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:

- For NCRAD/UNTHSC: Centrifuge capable of ≥ 2000 x g with refrigeration to 4°C
- For IU Path Lab: Centrifuge capable of 1300 x g 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**

**ABC-DS Blood-Based Biomarker Collection Schedule for DS Participants and Sibling Controls:**

<table>
<thead>
<tr>
<th>All visits</th>
<th>Serum</th>
<th>Plasma</th>
<th>DNA</th>
<th>RNA</th>
<th>Karyotyping₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHIP TO:</td>
<td>NCRAD &amp; UNTHSC</td>
<td>NCRAD &amp; UNTHSC</td>
<td>NCRAD</td>
<td>NCRAD</td>
<td>NCRAD</td>
</tr>
</tbody>
</table>

₁DS Participants only (if needed)
Clinical Labs Blood Collection Schedule for DS Participants ONLY

<table>
<thead>
<tr>
<th></th>
<th>Serum – Free T4, Thyroid, Triiodothyronine, TSH, Vit B12, ATA Preparation</th>
<th>Vit D, BMP, Lytes, Lipid Preparation</th>
<th>CBC Preparation</th>
<th>A1C Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>SHIP TO:</td>
<td>IU PATH LAB</td>
<td>IU PATH LAB</td>
<td>IU PATH LAB</td>
<td>IU PATH LAB</td>
</tr>
</tbody>
</table>

Whole blood is collected for the main study protocol (NCRAD and UNTHSC) in up to four different types of tubes (2) 5mL gold-top serum separator tube, 4mL green-top Sodium Heparin (NaHep) tube, (2) 10mL lavender-top EDTA tube and a 2.5 mL PAXgene™ tube. Whole blood is collected for the Clinical Labs (IU Path Lab) in up to three different types of tubes (1) 5 mL orange-top serum separator tube, (1) 5mL gold-top serum separator tube and (1) 3mL lavender-top EDTA tube.

**NCRAD and UNTHSC**

- The 10mL EDTA is processed locally into plasma and buffy coat fractions. They are then aliquoted, frozen at the study site and shipped to NCRAD and UNTHSC.
- The (2) 5mL gold top serum separator tubes are processed locally into serum fractions. They are then aliquoted, frozen at the study site and shipped to NCRAD and UNTHSC.
- The Sodium Heparin tubes are shipped to NCRAD on the day of the participant visit if karyotyping is needed (Monday through Thursday only).
- The PAXgene™ tube is frozen locally without further processing and shipped to NCRAD.

**IU Path Lab-Clinical Labs**

- The 5mL orange-top serum tube for Free T4, Thyroid, Triiodothyronine, TSH, Vit B12, ATA Preparation is processed locally into serum and shipped refrigerated to IU Path Lab on the day of the participant visit.
- The 5mL gold-top serum tube for Vit D, BMP, Lytes and Lipid Preparation is processed locally into serum and shipped refrigerated to IU Path Lab on the day of the participant visit.
- The 3mL lavender-top EDTA tube of whole blood for A1C and CBC Preparation is shipped refrigerated to IU Path Lab on the day of the participant visit.
**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.**

**If consent to bank with NCRAD is not obtained, only draw 1 EDTA (Lavender-Top) Blood Collection Tube (10 mL) and 1 Serum Separator (Gold-Top) Blood Collection Tube (5 mL). Send 2 serum aliquots and 17 plasma aliquots to UNTHSC. If consent for genetic research is not obtained, do not send buffy coats to NCRAD, since buffy coats are the source of DNA for said genetic research.**

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 following tables.

### 4.2.1 Re-Draw Instructions and Timeframes

Sample Collection-Blood eCRF is a log form. Select ‘Add a new record’ to enter a record. Enter one record per Date of Collection and specify samples collected. At least one sample type must be marked as collected on this date to successfully submit the form.

If a re-draw is necessary and occurs BETWEEN TWO VISITS, add a new record in the visit PRIOR to the re-draw timeframe, making sure to include the re-draw date of collection and Kit Number. If a sample was missed during a regularly scheduled visit, but a sample was collected PRIOR to NEXT scheduled visit, enter in the EDC as a re-draw. Also, provide reason for re-draw in the comments section.

For ABC-DS, the re-draw timeframe is as follows:

- For all visits, the re-draw timeframe will be up to 3 months prior and 3 months after the expected visit date.
## 4.3 Biospecimen Collection Chart
Research Blood Collection for DS Participants and Sibling Controls

<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>
<th>Tubes to UNTHSC</th>
<th>Ship</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>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>SERUM: 0.5 mL cryovials</td>
<td>21</td>
<td>0.25 mL serum aliquot per 0.5 mL siliconized cryovial (clear cap)</td>
<td>19</td>
<td>2</td>
<td>Frozen</td>
</tr>
<tr>
<td>Whole blood for Karyotyping*</td>
<td>Sodium Heparin (Green-Top) Blood Collection Tube (4 mL)</td>
<td>1</td>
<td>N/A</td>
<td>1</td>
<td>0</td>
<td>Ambient</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>2</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>PLASMA: 0.5 mL cryovials</td>
<td>41</td>
<td>0.25 mL plasma aliquot per 0.5 mL siliconized cryovial (clear cap)</td>
<td>24</td>
<td>17</td>
<td>Frozen</td>
</tr>
<tr>
<td></td>
<td>BUFFY COAT: 2.0 mL cryovials</td>
<td>2</td>
<td>1 mL buffy coat aliquot per 2.0 mL cryovial (BLUE CAP)</td>
<td>2</td>
<td>0</td>
<td>Frozen</td>
</tr>
<tr>
<td>Whole blood for RNA extraction</td>
<td>PAXgene™ Blood Collection Tube (2.5 mL)</td>
<td>1</td>
<td>N/A</td>
<td>1</td>
<td>0</td>
<td>Frozen</td>
</tr>
</tbody>
</table>

*DS participants only
### Clinical Labs Blood Collection for DS Participants ONLY

<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 IU Path Lab</th>
<th>Ship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood for isolation of serum</td>
<td>Serum Separator (Orange-Top) Blood Collection Tube (5 mL)</td>
<td>1</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>SERUM: 2.0 mL cryovials</td>
<td>2</td>
<td>1.0 mL serum aliquot per 2.0mL cryovial (CLEAR CAP)</td>
<td>2</td>
<td>Refrigerated</td>
</tr>
<tr>
<td>Serum Separator (Gold-Top) Blood Collection Tube (5 mL)</td>
<td>1</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SERUM: 2.0 mL cryovials</td>
<td>2</td>
<td>1.0 mL serum aliquot per 2.0mL cryovial (RED CAP)</td>
<td>2</td>
<td>Refrigerated</td>
</tr>
<tr>
<td>Whole Blood for CBC Preparation</td>
<td>EDTA (Lavender-Top) Blood Collection Tube (3 mL)</td>
<td>1</td>
<td>N/A</td>
<td>1</td>
<td>Refrigerated</td>
</tr>
<tr>
<td>Whole Blood for A1C Preparation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the IU Path Lab form (Appendix D). Submit a copy to IU Path Lab 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 serum/plasma/buffy coat/CSF aliquots, as well as partially completed shipping labels to send materials to UNTHSC, NCRAD and the IU Path Lab. Kit Number Labels, Site and BDS 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.

ABC-DS Blood Kit for DS Participants

<table>
<thead>
<tr>
<th>Quantity</th>
<th>ABC-DS Blood-Based Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PAXgene™ Blood Collection Tube (2.5 mL)</td>
</tr>
<tr>
<td>2</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>2</td>
<td>15 mL conical</td>
</tr>
<tr>
<td>41</td>
<td>Siliconized cryovial tube (0.5 mL) with clear cap</td>
</tr>
<tr>
<td>21</td>
<td>Siliconized cryovial tube (0.5 mL) with clear cap</td>
</tr>
<tr>
<td>2</td>
<td>Cryovial tube (2.0 mL) with blue cap</td>
</tr>
<tr>
<td>3</td>
<td>Disposable graduated transfer pipette (1 mL)</td>
</tr>
<tr>
<td>63</td>
<td>Pre-printed Collection and Aliquot Tube Label</td>
</tr>
<tr>
<td>5</td>
<td>Pre-printed Kit Number Label</td>
</tr>
<tr>
<td>5</td>
<td>Labels for handwritten Site and BDS ID</td>
</tr>
<tr>
<td>1</td>
<td>3”x5” self-seal bubble bag</td>
</tr>
<tr>
<td>1</td>
<td>Resealable bag</td>
</tr>
<tr>
<td>1</td>
<td>Cryobox 81-cell</td>
</tr>
<tr>
<td>1</td>
<td>Microcentrifuge tube box (holds up to 25 microcryovials)</td>
</tr>
</tbody>
</table>
### ABC-DS Blood Kit for Sibling Controls

<table>
<thead>
<tr>
<th>Quantity</th>
<th>ABC-DS Blood-Based Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PAXgene™ Blood Collection Tube (2.5 mL)</td>
</tr>
<tr>
<td>2</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>2</td>
<td>15 mL conical</td>
</tr>
<tr>
<td>41</td>
<td>Siliconized cryovial tube (0.5 mL) with clear cap</td>
</tr>
<tr>
<td>21</td>
<td>Siliconized cryovial tube (0.5 mL) with clear cap</td>
</tr>
<tr>
<td>2</td>
<td>Cryovial tube (2.0 mL) with blue cap</td>
</tr>
<tr>
<td>3</td>
<td>Disposable graduated transfer pipette (1 mL)</td>
</tr>
<tr>
<td>63</td>
<td>Pre-printed Collection and Aliquot Tube Label</td>
</tr>
<tr>
<td>5</td>
<td>Pre-printed Kit Number Label</td>
</tr>
<tr>
<td>5</td>
<td>Labels for handwritten Site and BDS ID</td>
</tr>
<tr>
<td>1</td>
<td>3”x5” self-seal bubble bag</td>
</tr>
<tr>
<td>1</td>
<td>Resealable bag</td>
</tr>
<tr>
<td>1</td>
<td>Cryobox 81-cell</td>
</tr>
<tr>
<td>1</td>
<td>Microcentrifuge tube box (holds up to 25 microcryovials)</td>
</tr>
</tbody>
</table>

### ABC-DS Blood Kit for Clinical Labs

<table>
<thead>
<tr>
<th>Quantity</th>
<th>ABC-DS Blood-Based Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Serum Separator (Orange-Top) Blood Collection Tube (5 mL)</td>
</tr>
<tr>
<td>1</td>
<td>EDTA (Lavender-Top) Blood Collection Tube (3 mL)</td>
</tr>
<tr>
<td>1</td>
<td>Serum Separator (Gold-Top) Blood Collection Tube (5 mL)</td>
</tr>
<tr>
<td>2</td>
<td>Cryovial tube (2.0 mL) with clear cap</td>
</tr>
<tr>
<td>2</td>
<td>Cryovial tube (2.0 mL) with red cap</td>
</tr>
<tr>
<td>2</td>
<td>Disposable graduated transfer pipette (3 mL)</td>
</tr>
<tr>
<td>8</td>
<td>Labels for handwritten Site BDS ID and DOB</td>
</tr>
<tr>
<td>1</td>
<td>Resealable bag</td>
</tr>
<tr>
<td>1</td>
<td>Microcentrifuge tube box (holds up to 25 microcryovials)</td>
</tr>
<tr>
<td>3</td>
<td>Resealable tube pouches, on roll</td>
</tr>
<tr>
<td>1</td>
<td>Pre-printed UPS Return Airbill addressed to IU Path Lab</td>
</tr>
<tr>
<td>1</td>
<td>Biohazard bags with absorbent sheet (small)</td>
</tr>
<tr>
<td>1</td>
<td>X-Small insulated shipper</td>
</tr>
<tr>
<td>2</td>
<td>Cold brick packs</td>
</tr>
<tr>
<td>1</td>
<td>Brown corrugated box</td>
</tr>
<tr>
<td>5</td>
<td>Air cushions</td>
</tr>
<tr>
<td>1</td>
<td>Warning label packet</td>
</tr>
<tr>
<td>1</td>
<td>Round fluorescent “ABC-DS Study” sticker</td>
</tr>
<tr>
<td>1</td>
<td>Large rectangular fluorescent “ABC-DS Study” sticker</td>
</tr>
</tbody>
</table>
### ABC-DS 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 (large)</td>
</tr>
<tr>
<td>5</td>
<td>Biohazard bag with Absorbent sheet (small)</td>
</tr>
<tr>
<td>2</td>
<td>Shipping airbill</td>
</tr>
<tr>
<td>2</td>
<td>Resealable bag</td>
</tr>
<tr>
<td>2</td>
<td>Shipping box/Styrofoam container</td>
</tr>
<tr>
<td>2</td>
<td>Warning label packet with dry ice sticker</td>
</tr>
</tbody>
</table>

### ABC-DS Ambient Blood Shipping Supply Kit

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Ambient Shipping Kit Components for Blood-Based Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Plastic biohazard bag with absorbent sheet</td>
</tr>
<tr>
<td>1</td>
<td>Small IATA shipping box with insulated cooler</td>
</tr>
<tr>
<td>1</td>
<td>Small refrigerant pack</td>
</tr>
<tr>
<td>1</td>
<td>Aqui-Pak 6 tube absorbent pouch</td>
</tr>
<tr>
<td>1</td>
<td>UN3373 Biological Substance Category B label</td>
</tr>
<tr>
<td>1</td>
<td>List of contents card</td>
</tr>
<tr>
<td>1</td>
<td>FedEx return airbill</td>
</tr>
<tr>
<td>1</td>
<td>FedEx Clinic Pak</td>
</tr>
</tbody>
</table>

### Blood Supplemental Supply Kit

<table>
<thead>
<tr>
<th>Quantity</th>
<th>ABC-DS Blood-Based Kit Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>PAXgene™ Blood Collection Tube (2.5 mL)</td>
</tr>
<tr>
<td>10</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>5</td>
<td>Serum Separator (Orange-Top) Blood Collection Tube (5 mL)</td>
</tr>
<tr>
<td>5</td>
<td>EDTA (Lavender-Top) Blood Collection Tube (3 mL)</td>
</tr>
<tr>
<td>10</td>
<td>15 mL conical</td>
</tr>
<tr>
<td>50</td>
<td>Siliconized cryovial tube (0.5 mL) with clear cap</td>
</tr>
<tr>
<td>25</td>
<td>Siliconized cryovial tube (0.5 mL) with clear cap</td>
</tr>
<tr>
<td>10</td>
<td>Cryovial tube (2.0 mL) with blue cap</td>
</tr>
<tr>
<td>15</td>
<td>Disposable graduated transfer pipette (3 mL)</td>
</tr>
<tr>
<td>10</td>
<td>Labels for handwritten Site and BDS ID</td>
</tr>
<tr>
<td>10</td>
<td>3”x5” self-seal bubble bag</td>
</tr>
<tr>
<td>10</td>
<td>Resealable bag</td>
</tr>
<tr>
<td>5</td>
<td>Cryobox 81-cell</td>
</tr>
<tr>
<td>5</td>
<td>Microcentrifuge tube box (holds up to 25 microcryovials)</td>
</tr>
<tr>
<td>5</td>
<td>Plastic Biohazard bag with absorbent sheet (large)</td>
</tr>
<tr>
<td>5</td>
<td>Biohazard bag with Absorbent sheet (small)</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>PAXgene™ Blood Collection Tube (2.5 mL)</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>
<tr>
<td>By Request</td>
<td>Sodium Heparin (Green-Top) Blood Collection Tube (4 mL)</td>
</tr>
<tr>
<td>By Request</td>
<td>15 mL conical</td>
</tr>
<tr>
<td>By Request</td>
<td>Serum Separator (Orange-Top) Blood Collection Tube (5 mL)</td>
</tr>
<tr>
<td>By Request</td>
<td>EDTA (Lavender-Top) Blood Collection Tube (3 mL)</td>
</tr>
<tr>
<td>By Request</td>
<td>Serum (Gold-Top) Blood Collection Tube (5 mL)</td>
</tr>
<tr>
<td>By Request</td>
<td>Siliconized cryovial tube (0.5 mL) with clear cap</td>
</tr>
<tr>
<td>By Request</td>
<td>Siliconized cryovial tube (0.5 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>Disposable graduated transfer pipette</td>
</tr>
<tr>
<td>By Request</td>
<td>Labels for handwritten Site and BDS ID</td>
</tr>
<tr>
<td>By Request</td>
<td>3”x5” self-seal bubble bag</td>
</tr>
<tr>
<td>By Request</td>
<td>Resealable bag</td>
</tr>
<tr>
<td>By Request</td>
<td>Cryobox 81-cell</td>
</tr>
<tr>
<td>By Request</td>
<td>Microcentrifuge tube box (holds up to 25 microcryovials)</td>
</tr>
<tr>
<td>By Request</td>
<td>Plastic Biohazard bag with absorbent sheet (large)</td>
</tr>
<tr>
<td>By Request</td>
<td>Biohazard bag with Absorbent sheet (small)</td>
</tr>
<tr>
<td>By Request</td>
<td>FedEx return airbill</td>
</tr>
<tr>
<td>By Request</td>
<td>Resealable bag</td>
</tr>
<tr>
<td>By Request</td>
<td>Shipping box/Styrofoam container</td>
</tr>
<tr>
<td>By Request</td>
<td>Warning label packet with dry ice sticker</td>
</tr>
<tr>
<td>By Request</td>
<td>Small IATA shipping box with insulated cooler</td>
</tr>
<tr>
<td>By Request</td>
<td>Small refrigerant pack</td>
</tr>
<tr>
<td>By Request</td>
<td>Aqui-Pak 6 tube absorbent pouch</td>
</tr>
<tr>
<td>By Request</td>
<td>UN3373 Biological Substance Category B label</td>
</tr>
<tr>
<td>By Request</td>
<td>List of contents card</td>
</tr>
<tr>
<td>By Request</td>
<td>Shipping Clin Pak</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 ABC-DS 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. For international sites, please allow THREE 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. There are 2 options for blood draw order:

**Draw Order – Option 1 (PREFERRED):**

**Research collection (Day 1):**

- 1. Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 2
- 2. Sodium Heparin (Green-Top) Blood Collection Tube (4 mL) for Karyotyping (DS Participants only, as needed)
- 3. EDTA (Lavender-Top) Blood Collection Tube (10 mL) for DNA and Plasma x 2
- 4. PAXgene™ Blood Collection Tube (2.5 mL) for RNA

**Clinical labs collection (Day 2):**

- 1. Serum Separator (Orange-Top) Blood Collection Tube (5 mL) for Serum x 1
- 2. Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 1
- 3. EDTA (Lavender-Top) Blood Collection Tube (3mL) for hematology

**Draw Order – Option 2:**

**Collection – Research and Clinical Labs on same day/visit:**

- 1. Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 2 (NCRAD)
- 2. Serum Separator (Orange-Top) Blood Collection Tube (5 mL) for Serum x 1 (IU Path Lab)
- 3. Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 1 (IU Path Lab)
- 4. Sodium Heparin (Green-Top) Blood Collection Tube (4 mL) for Karyotyping (DS Participants only, as needed) (NCRAD)
- 5. EDTA (Lavender-Top) Blood Collection Tube (10 mL) for DNA and Plasma x 2 (NCRAD)
- 6. EDTA (Lavender-Top) Blood Collection Tube (3 mL) for hematology (IU Path Lab)
- 7. PAXgene™ Blood Collection Tube (2.5 mL) for RNA x 1 (NCRAD)

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 BDS ID Label
4. Site BDS ID and DOB Labels

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 for UNTHSC and NCRAD.

** Extra Kit Number Label included in each Blood kit in case NaHep tube requested for karyotyping.

The **Collection and Aliquot Tube Labels** for blood derivatives and CSF are placed on all collection and aliquot tubes for UNTHSC and NCRAD. **Note:** Aliquot Tube Labels for Plasma and Serum are color-coded to replace cap stickers.

The **Site and BDS ID Labels** are placed on all blood collection tubes for UNTHSC and NCRAD.

The **Site BDS ID and DOB Labels** are placed on all tubes for IU Path Lab.
**Important Note**

Each collection tube for NCRAD and UNTHSC will contain two labels: the Collection and Aliquot Tube Label and the Site and BDS 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 BDS ID label below.

Each NaHep tube for Karyotyping will contain two labels: the Site and BDS ID label and the kit number label.

---

NaHep Blood Collection Tube (4 mL)  
Serum Separator (Gold-Top) Blood Collection Tube (5 mL)  
EDTA (Lavender-Top) Blood Collection Tube (10 mL)  
PAXgene™ Blood Collection Tube (2.5 mL)

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**Important Note**

Each collection tube for IU Path Lab will contain one label: the Site BDS ID and DOB Label. Make sure the subject’s DOB is filled in and matches the DOB written on the IU Path Lab Req Form (Appendix D).
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 and place the Site and BDS ID Labels on the collection tubes only (SST, NaHep, EDTA, RNA) BEFORE sample collection, processing, or freezing. These labels are in addition to the Collection and Aliquot Tube Labels. **DO NOT** place Site and BDS 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.
• **Important Note:** Aliquot Tube Labels for Plasma and Serum are color-coded to replace color coded cap stickers. Cap stickers were causing issues with robotic freezer storage.

• 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:

- **ABC-DS MOP Training**
- **Frozen Shipping**
- **Ambient Shipping**
6.3 Filling Aliquot Tubes (Plasma and Serum)

In order to ensure that UNTHSC and NCRAD receive 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. 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 colored label stripes are used for the cryovial tubes. The chart below summarizes the association between cap color, colored label strips and type of cryovial.
<table>
<thead>
<tr>
<th>Color Coding</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear Cap Siliconized / Purple strip on label</td>
<td>Plasma</td>
</tr>
<tr>
<td>Clear Cap Siliconized / Red strip on label</td>
<td>Serum</td>
</tr>
<tr>
<td>Blue Cap</td>
<td>Buffy Coat</td>
</tr>
<tr>
<td>Clear Cap</td>
<td>Serum – Clinical Labs from Orange-Top Tube</td>
</tr>
<tr>
<td>Red Cap</td>
<td>Serum – Clinical Labs from Gold-Top Tube</td>
</tr>
</tbody>
</table>

**Color Coding Examples:**
- Clear Cap Siliconized / Purple strip on label
- Blue Cap
- Clear Cap
- Red Cap

**Sample Types with Examples:**
- Plasma: Clear Cap Siliconized / Purple strip on label
- Serum: Clear Cap Siliconized / Red strip on label
- Buffy Coat: Blue Cap
- Serum: Clear Cap
- Serum: Red Cap

**Sample Identification:**
- Kit #: 440303

**Sample Types with Examples:**
- Plasma
- Serum

Version (9.2022)
6.4 Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 2

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 BDS 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 Aliquot “SERUM” Tube Labels with color-coded red strip on the (21) 0.5 mL siliconized cryovial tubes with clear caps.

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.
a. **EXCEPTION:** If field-draw, allow blood to clot at room temperature before placing on wet ice, upright on rack and transferring to lab for further processing. Please check “Yes” box on sample form *(Appendix B)* if field-draw and make note on Appendix F. If processing takes longer than 2 hours, please make note on both forms.

7. After 30 minutes of clotting, centrifuge the collection tubes for 10 minutes at 2000 RCF (x g) at 4°C. It is critical that the tubes 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. **EXCEPTION:** If field-draw, place tube on wet ice until you reach the lab for processing.
- Record time aliquoted on the Biological Sample Shipment and Notification Form

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**SST immediately after blood draw.**

**SST immediately following the centrifuge.**
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 plasma from the 15 mL conical tube into the pre-labeled siliconized “SERUM” cryovials with color-coded red strip. 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 with color-coded red strip. 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 2 of labeled cryovials in the 25-cell cryovial box for UNTHSC (these samples take priority – if only 2 are collected, only send to UNTHSC) and place the rest (up to 19 cryovials) in the 81-cell cryovial box for NCRAD and place on dry ice. Transfer to -80°C Freezer when possible. Store all samples at -80°C until shipped to UNTHSC and NCRAD on dry ice.

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.
Serum Preparation (5 ml Gold-Top Tube) X 2

- Store tubes at room temperature.
- 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 2 hours 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.

Up to 19 sent to NCRAD
Up to 2 sent to UNTHSC

- Aliquot 0.25 ml into each labeled 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 Sodium Heparin (Green-Top) Blood Collection Tube (4 mL) for karyotyping

***Important Note***
If karyotyping has already been done for the participant, please check “Yes” on the Biological Sample and Shipment Notification Form (Appendix B).

Once drawn, Sodium Heparin tubes MUST be shipped to NCRAD on the day of collection via overnight delivery. This is to ensure the specimen has the most viable cells available at extraction. These samples should only be collected Monday-Thursday. Please DO NOT collect these samples on Fridays.

1. CRITICAL STEP: Store empty Sodium Heparin tube at room temperature, 64°F – 77°F (18°C to 25°C) before use.

2. Place completed BDS ID Label and Kit Number Label on the green-top NaHep tube.

3. Using a blood collection set and a holder, collect blood into the 4 mL Sodium Heparin tubes using your institution’s recommended procedure for standard venipuncture technique.

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

4. Immediately after blood collection, gently invert the tubes 8-10 times to mix sample. If field-draw, keep at room temperature until shipping.

5. Seal the Sodium Heparin tubes in the ambient shipment kit.
6. Fill in the ABC-DS BDS ID and the NaHep tube volume in section 2 of the *Constitutional (Blood) Test Requisition Form (Appendix E)* and fill out the NaHep tube volume on the *Biological Sample Request Form (Appendix B)* as well. Print out this form and ship with the Sodium Heparin tube.

7. Ship the unprocessed tubes ambient to NCRAD. **Samples must be shipped the same day as collection. Samples must be received the following day after collection. Do NOT draw or ship ambient samples on Friday. Only Monday-Thursday collection and same day shipping.**
6.6 EDTA (Lavender-Top) Blood Collection Tube (10 mL) for Plasma and Buffy Coat x 2

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 BDS ID Label and pre-printed “PLASMA” Collection and Aliquot Tube Label on the lavender-top EDTA tube (2 x 10 mL). Place pre-printed Aliquot “PLASMA” Tube Labels with color-coded purple strip on the (41) 0.5 mL siliconized cryovial tubes with clear caps. Place pre-printed “BUFFY COAT” Collection and Aliquot Tube Label on the (2) 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) x 2 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 tubes 8-10 times.

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

   a. **If field-draw,** keep the samples on wet ice until you reach your destination. Please check “Yes” box on sample form (Appendix B) if field-draw and make note on Appendix F.

   ➤ 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. **If field-draw, place tube on wet ice until you reach the lab for processing.**

   ➤ Record time aliquoted on the Biological Sample Shipment and Notification Form.

8. 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 buffy coat and packed red blood cells at the bottom of the tube (see below). Transfer plasma from both lavender-top EDTA tubes into the single 15mL conical tube. Mix the plasma by gently inverting the conical tube 3 times.

9. Using a pipette, transfer plasma from the 15 mL conical tube into the pre-labeled siliconized “PLASMA” cryovials with color-coded purple strip. Aliquot 0.25 mL per cryovial (total vials = 35-41 with 0.25 mL each). The EDTA tube should yield, on average, 5 mL of plasma for a total of 35-41 0.5 mL siliconized cryovial tubes per subject with 0.25 mL per cryovial tube. Be sure to only place **plasma** in siliconized cryovials labeled with the “PLASMA” label with color-coded purple strip. 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.**
NOTE: When pipetting plasma from the plasma tube into the 15 mL conical tube, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.
10. Place 17 of the labeled cryovials in the 25-cell cryovial box for UNTHSC (these samples take priority – if only 17 are collected, only send to UNTHSC) and place the rest (up to 24 cryovials) in the 81-cell cryovial box for NCRAD and place on dry ice. Transfer to -80°C Freezer when possible. Store all samples at -80°C until shipped to UNTHSC and NCRAD on dry ice.

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 blue cap using a micropipette. The buffy coat aliquots are expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovials with the blue cap and “BUFFY COAT” label.

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

13. Place 2 of the labeled buffy coat cryovials in the 81-cell cryovial box for NCRAD and place on dry ice. Transfer to -80°C Freezer when possible. Store all samples at -80°C until shipped to UNTHSC and NCRAD on dry ice.

***Important Note***
SNP fingerprint is also obtained from every DNA sample, to be compared longitudinally across study visits to identify any subject/sample mix-ups. Apolipoprotein E (APOE) genotype is generated in-house as part of this fingerprint assay.
Plasma and Buffy Coat Preparation (10 mL Lavender-Top Tube) x 2

Step One
- Store tubes at room temperature.
- Place completed Sich and BDS ID Label and Collection and Aliquot "PLASMA" Tube Labels on 10 mL Lavender-Top tubes prior to blood draw.
- Place pre-printed Aliquot "PLASMA" Tube Labels with color-coded purple strip on the (41) 0.5 mL siliconized cryovial tubes with clear caps prior to blood draw.
- Place pre-printed Aliquot "BUFFY COAT" Tube Labels on the (2) blue cap cryovials prior to blood draw.

Step Two
- Collect blood in (2) 10 mL EDTA tubes allowing blood to flow for 10 seconds and ensure blood flow has stopped.

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

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

Step Five
- 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 Six
- Using a clean transfer pipette, transfer Plasma from both 10 mL EDTA tubes to the 15 mL conical tube.
- Invert 15 mL conical tube 3 times to mix the Plasma.

Step Seven
- Aliquot 0.25 mL into each labeled cryovial tube.
- If a residual aliquot is created, document specimen number on Sample Notification Form.
- Store Plasma aliquots at -80°C until shipment.

Step Eight
- Using a clean pipette tip aliquot buffy coat layer (may have residual plasma and RBCs) into labeled cryovials with blue cap.
- Store Buffy Coat aliquots at -80°C until shipment.
6.7 2.5 mL PAXgene™ Tube for RNA x 1

***Important Note***
In the event the PAXgene™ tube is the only tube being collected at the visit, a 4mL serum discard tube must be drawn first.

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

1. Place filled-out Site and BDS ID Label and Collection and Aliquot “RNA” Tube Label on the PAXgene™ tube prior to blood draw; no processing is required for this tube. **The single tube is to be shipped to NCRAD frozen, without processing at the collection site.**

2. **CRITICAL STEP:** Store PAXgene™ RNA Tubes at room temperature 64°F – 77°F (18°C to 25°C) before use.

3. Using a blood collection set and a holder, collect blood into the PAXgene™ RNA Tube using your institution’s recommended procedure for standard venipuncture technique.

   **The following techniques shall be used to prevent possible backflow:**
   
   a. Place participant’s arm in a downward position.
   b. Hold tube in a vertical position, below the participant’s arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into 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 the tube before removing the tube from the holder.** The PAXgene™ RNA Tube with its vacuum is designed to draw 2.5 mL of blood into the tube.

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

6. Place the PAXgene™ RNA tube upright in a WIRE rack and transfer the PAXgene™ RNA tube to a -80°C freezer. Keep the PAXgene™ RNA Tube in -80°C freezer for storage until you ship on dry ice to NCRAD. **If field-draw,** transfer tube upright in a WIRE rack at room temperature until storage in a -
80°C freezer. Complete remainder of the Biological Sample and Shipment Notification Form (Appendix B). Please check “Yes” box on sample form (Appendix B) if field-draw and make note on Appendix F.
RNA Preparation (2.5mL PAXgene™ Tube) x 1

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

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

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

**Step Four**
- Store tubes at -80°C in a wire rack until shipment.
6.8 Clinical Labs – IU Path Lab

6.8.1 Serum Separator (Orange-Top) Blood Collection Tube (5 mL) for Serum x 1

Whole Blood Collection for Isolation of Serum: Serum Separator (Orange-Top) Blood Collection Tube (5 mL) for processing of serum aliquots for Free T4, Thyroid, Triiodothyronine, TSH, Vit B12, ATA Preparation.

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

2. Place completed Site BDS ID and DOB Label on the Serum Separator (Orange-Top) Blood Collection Tubes (1 x 5 mL) and (2) 2.0 mL cryovial tubes with clear caps.

3. Using a blood collection set and a holder, collect blood into Serum Separator (Orange-Top) Blood Collection Tubes (1 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 IU Path Lab Req Form (Appendix D). 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) tube 5 times.

6. CRITICAL STEP: For best results, serum samples should be spun within 1 hour from the time of collection. EXCEPTION: If field-draw, processing must be completed within 2 hours from time of collection. Place tube on rack in upright position during transfer to lab with cold
packs until able to process. Please note on the IU Path Lab form (Appendix D) that it is a field-draw and the time it takes to process the samples.

7. Centrifuge the collection tube for 10 minutes at 1300 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 1300 x g
   - Serum samples need to be spun within 1 hour of collection.
Free T4, Thyroid, Triiodothyronine, TSH, Vit B12, ATA Preparation (1 X 5 ml Orange Top Tubes)

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

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

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

**Step Four**
- Within 60 minutes of blood draw, centrifuge samples at 1500 x g at 4°C for 10 minutes.

**Step Five**
- Label clear cap cryovial tubes with preprinted labels.
- Aliquot 1.0ml of serum into each cryovial tube.
- Store serum aliquots in refrigerator until shipment.
6.8.2 Serum Separator (Gold-Top) Blood Collection Tube (5 mL) for Serum x 1

Whole Blood Collection for Isolation of Serum: Serum Separator (Gold-Top) Blood Collection Tube (5 mL) (for processing of serum aliquots for Vit D, BMP, Lytes and Lipid Preparation)

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

2. Place completed Site BDS ID and DOB Label on the Serum Separator (Gold-Top) Blood Collection Tubes (1 x 5 mL) and (2) 2.0 mL cryovial tubes with red caps.

3. Using a blood collection set and a holder, collect blood into Serum Separator (Gold-Top) Blood Collection Tubes (1 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 IU Path Lab Req Form (Appendix D). 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) 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. For best results, serum samples should be spun within 1 hour from the time of collection. EXCEPTION: If field-draw, processing must be completed within 2 hours from time of collection. Place tube on rack in vertical position during transfer to lab with cold packs until able
to process. Please note on the IU Path Lab form (Appendix D) that it is a field-draw and the time it takes to process the samples.

7. After 30 minutes of clotting, centrifuge the collection tube for 10 minutes at 1300 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 1300 x g
   - Serum samples need to be spun within 1 hour of collection.
Vit D, BMP, Lytes and Lipid Preparation (1 X 5 ml Gold Top Tube)

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

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

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

Step Four
- Allow blood to clot for 30 minutes.
- Within 60 minutes of blood draw, centrifuge samples at 1300 x g at 4°C for 10 minutes.

Step Five
- Label red cap cryovial tubes with preprinted labels.
- Aliquot 1.0ml of serum into each cryovial tube.
- Store serum aliquots in refrigerator until shipment.
6.8.3 EDTA (Lavender-Top) Blood Collection Tube (3 mL) x 1

Whole Blood Collection for CBC and A1C Preparation: EDTA (Lavender-Top) Blood Collection Tube (3 mL)

1. Place filled-out Site BDS ID and DOB Label on the EDTA tube prior to blood draw; no processing is required for this tube. The single tube is to be shipped to IU Path Lab refrigerated, without processing at the collection site.

2. CRITICAL STEP: Store EDTA Tube at room temperature 64°F - 77°F (18°C to 25°C) before use.

3. Using a blood collection set and a holder, collect blood into the EDTA (Lavender-Top) Blood Collection Tube (3 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 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 the tube before removing the tube from the holder. The EDTA (Lavender-Top) Blood Collection Tube (3 mL) with its vacuum is designed to draw 3.0 mL of blood into the tube.

5. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA (Lavender-Top) Blood Collection Tube (3 mL) Tube 8 – 10 times.

6. Store EDTA (Lavender-Top) Blood Collection Tube (3 mL) in refrigerator until shipment. If field draw, keep tube on cold packs during transfer to lab. Store tube in refrigerator until shipment. Please note on the IU Path Lab form (Appendix D) that it is a field-draw.
CBC and A1C Preparation (1 x 3ml EDTA Purple Top Tube)

Step One
- Store tubes at room temperature.
- Label tubes prior to blood draw.

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

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

Step Four
- Store whole blood tube in refrigerator 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 UNTHSC and NCRAD. See page 11 of the manual for re-draw instructions.

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 UNTHSC and 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
   d. If samples are hemolyzed (see below), please do not send.
2. If the biospecimens at a scheduled visit are not collected:
   a. Contact the ABC-DS Monitor 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.
      i. If samples were unable to be drawn, please draw the Sodium
         Heparin (Green-Top) Tube for Karyotyping during the next visit (as
         needed).

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.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Processing/ Aliquoting</th>
<th>Tubes to NCRAD</th>
<th>Tubes to UNTHSC</th>
<th>Ship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood for RNA extraction</td>
<td>N/A</td>
<td>1</td>
<td>0</td>
<td>Frozen</td>
</tr>
<tr>
<td>Whole blood for isolation of serum</td>
<td>0.25 mL serum aliquot per 0.5 mL siliconized cryovial (clear cap)</td>
<td>19</td>
<td>2</td>
<td>Frozen</td>
</tr>
<tr>
<td>Whole blood for isolation of plasma &amp; Buffy coat (for DNA extraction)</td>
<td>0.25 mL plasma aliquot per 0.5 mL siliconized cryovial (clear cap)</td>
<td>24</td>
<td>17</td>
<td>Frozen</td>
</tr>
<tr>
<td></td>
<td>1 mL Buffy coat aliquot per 2.0 mL cryovial (BLUE CAP)</td>
<td>2</td>
<td>0</td>
<td>Frozen</td>
</tr>
<tr>
<td>Whole blood for Karyotyping</td>
<td>N/A</td>
<td>1</td>
<td>0</td>
<td>Ambient</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 (if UW-Madison, 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:
     o Completed Biological Sample and Shipment Notification Form (Appendix B – also found on the NCRAD ABC-DS study page).
If email is unavailable please call NCRAD and do not ship until you’ve contacted and notified NCRAD coordinators about the shipment in advance.

- Please include the tracking number in the body of the email.

**Notify UNTHSC of shipment** by emailing UNTHSC Lab Manager at: Tori.Como@unthsc.edu

- Attach the following to the email:
  - Completed UNTHSC Intake Form (**Appendix F** – also found on the [NCRAD ABC-DS study page](#)) and the UNTHSC Import Batch Form (**Appendix G**):
    - Aliquot barcodes need to be listed on the UNTHSC Import Batch Form (**Appendix G**). NCRAD will send an Excel file with all aliquot barcodes included in each kit when kit supplies are shipped.
  - If email is unavailable please call UNTHSC and do not ship until you’ve contacted and notified UNTHSC Lab Manager about the shipment in advance.
  - Please include the tracking number in the body of the email.

3. Place all frozen labeled aliquots of serum, plasma and buffy coat in the cryovial cryoboxes.
   - **FOR NCRAD**: Each cryobox holds 81 samples and there will be approximately 55 cryovial samples being shipped to NCRAD. Please place serum, plasma, and buffy coat aliquots within one cryobox (19 serum, 24 plasma, and 2 buffy coats) per participant blood draw (see below).
   - **FOR UNTHSC**: Each cryobox holds 25 samples and there will be approximately 19 cryovial samples being shipped to UNTHSC. Please place plasma and serum aliquots within one cryobox (2 serum, 17 plasma) per participant blood draw (see below).
iii. Cryoboxes should contain all of the specimens from the same patient, per time point.

iv. **FOR NCRAD** - Batch shipping should be performed every 3 months or when specimens from 5 participants accumulates, whichever is sooner.

v. **FOR UNTHSC** – Batch shipping should be performed every 3 months or when specimens from 5 participants accumulate, whichever is sooner.

4. Label the outside of the cryoboxes with the kit number label.

   i. **FOR NCRAD** - Place serum, plasma and buffy coat aliquots within one cryobox. Place RNA tube in bubble wrap tube sleeve and place within the SAME biohazard bag. These biohazard bags are large enough to contain one cryobox and the RNA tube.

   ii. **FOR UNTHSC** – Place serum and plasma aliquots within one cryobox in a small biohazard bag.

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 (as pictured below).
8. Fully cover the cryoboxes with approximately 2 inches of dry ice.

9. 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.

10. Replace the lid on the Styrofoam carton. Place the completed Biological Sample and Shipment Notification Form for NCRAD or the UNTHSC Intake form and UNTHSC Import Batch form for UNTHSC in the package on top of the Styrofoam lid for each patient specimen. Close and seal the outer cardboard shipping carton with packing tape.

11. Complete the FedEx return airbill (if UW-Madison, follow UPS instructions provided at site) 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 sender and bill transportation costs to your site’s study FedEx account number.

12. 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:
d. Do not cover any part of this label with other stickers, including pre-printed address labels.

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

**IMPORTANT!**

| Complete the required fields on the FedEx return airbill and Class 9 Dry Ice label, or FedEx may reject or return your package. |

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

15. 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.

16. Use FedEx tracking to ensure the delivery occurs as scheduled and is received by NCRAD. Please notify NCRAD by email (alzstudy@iu.edu) and UNTHSC by email at UNTHSC Lab Manager by email (Tori.Como@unthsc.edu) that a shipment has been sent and include the FedEx tracking number in your email.
In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by NCRAD for each sample type. Investigators and clinical coordinators for each project are responsible to ensure the requested amounts of each fluid are collected to the best of their ability and that samples are packed with sufficient amounts of dry ice to avoid thawing in the shipment process.

8.2 Ambient Shipping Instructions

***Important Note***

For ambient Sodium Heparin (Green-Top) Blood Collection Tube (1 x 4 mL) shipments, include no more than one tube per shipping container and only include tube from one participant. The ambient NaHep sample must be shipped the day of blood draw.

The labeled, unprocessed, sodium heparin NaHep tube will be shipped to NCRAD as outlined below.
Ambient Sodium Heparin (Green-Top) Blood Collection Tube (4 mL) shipments should be considered as Category B UN3373 and as such must be tripled packaged and compliant with the IATA Packing Instructions 650. See the Latest Edition of the IATA Regulations for complete documentation.

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

8.2.1 NCRAD Packaging and Shipment Instructions (Ambient Shipments)

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

2. Contact FedEx (if UW-Madison, contact UPS) to confirm service is available and schedule package to be picked up.
3. Notify NCRAD of shipment by emailing NCRAD coordinators at: alzstudy@iu.edu
   a. Complete and attach the Constitutional (Blood) Test Requisition Form to the email. (See Appendix E for an example of the form)

4. Place filled and labeled sodium heparin (green-top) tube within the slots in the absorbent pad provided, and place into the plastic biohazard bag with absorbent sheet. Place the filled out Constitutional (Blood) Test Requisition Form (Appendix E) inside the biohazard bag as well.

5. Remove as much air as possible from the plastic biohazard bag and ensure the Kit Number Label and BDS ID Label are placed on the tube before sealing the bag according to the directions printed on the bag.

6. Place the sealed biohazard bag inside the cooler and place the refrigerant pack into the cooler on top of the filled biohazard bag.

7. Place the lid onto the cooler.
8. Place the cooler in the provided small IATA Shipping Box.

9. Close shipping box. Label the outside of the cardboard box with the enclosed UN3373 (Biological Substance Category B) label.

10. Place the closed, labeled shipping box within a Clinical Pak. **Seal the Clinical Pak.**

11. Place return airbill on the sealed Clinical Pak.
   a. Be sure to complete the return airbill with the following information:
      o Section 1, “From”: fill in the date, your name, and phone number.
      o Section 2, “Your Internal Billing Reference”: add any additional information required by your site.

12. Specimens should be sent to the below address via **FedEx Priority Overnight (or UPS Next Day Air)**. Ambient shipments should be sent Monday through Thursday.

   **ABC-DS at NCRAD**
   Indiana University School of Medicine
   351 West 10th Street
   TK-217
   Indianapolis, IN 46202

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

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

1. All international shipments will utilize the same packing requirements as specified in Section 8.1 (Frozen Shipping Instructions). Please note that UNTHSC will not be receiving international shipments. International samples specified for UNTHSC will need to be shipped to NCRAD and will then be forwarded to UNTHSC.
   a. International sites will receive a fluorescent label to adhere to the outside of the shipping container so it is clear that NCRAD should forward the international samples to UNTHSC.

2. Two components are necessary for international shipments:
   1. International FedEx return airbill
   2. International Commercial Invoice

3. NCRAD will provide an International FedEx return airbill to all International sites. However, these international sites are welcome to utilize the FedEx electronic system.
   a. Be sure to complete the FedEx return airbill with the following information:
      i. Section 1, From: Enter the date and your name, phone number, complete address, and FedEx account number.
      ii. Section 2, To: This information will be preprinted with NCRAD’s return address and phone number.
      iii. Section 3, Shipment information: This information does NOT replace a Commercial Invoice that is required for these shipments. Total Packages, Weight, and box dimensions are required. Be consistent between this International FedEx return airbill and the International Commercial Invoice.
         1. Do not declare the value of the shipment to be over $2,500. This would require additional paperwork (a Shipper’s Export Declaration form).
      iv. Section 4, Express Package Services: Please check FedEx Intl. Priority for both Frozen and Ambient Shipments. (Pictured)

      v. Section 5, Packaging: Please select “Other” for Frozen Shipments and “FedEx Pak” for Ambient Shipments.
vi. Section 6, Special Handling: Please leave blank.

vii. Section 7 and 8, Payment: Check Sender and bill transportation costs to your site’s study FedEx account number. Duties and Taxes will also be billed to the sender. If your site requests information to be included as reference, please complete Section 8.

viii. Section 9, Required Signature: This section must be signed by the sender or department representative.

b. International Commercial Invoice (See Appendix C – fillable online PDF here)

i. The International Commercial Invoice must be completed and placed with the International return airbill.

1. Include **ONE** original and **THREE** copies of this completed form with the FedEx return airbill.

ii. Complete “Shipped From” with your name, address, and any additional contact information.

iii. Complete “Shipped To, Consignee” with the NCRAD shipping address:

   **NCRAD**
   IU School of Medicine
   351 West 10th Street
   TK-217
   Indianapolis, IN 46202
   Phone: 1-800-526-2839

iv. Complete Number of Packages and Shipping weight to match the information recorded within the International FedEx return airbill.

v. Immediately below the shipping weight is a section asking for the Country of Origin, Description of Goods, Quantity, Unit Price, and Total Price. Please be as detailed as possible within this section (example pictured below).

<table>
<thead>
<tr>
<th>COUNTRY OF ORIGIN</th>
<th>DESCRIPTION OF GOODS</th>
<th>QUANTITY</th>
<th>UNIT PRICE</th>
<th>TOTAL PRICE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada, Vancouver</td>
<td>Non-Infectious, non-contagious, human Plasma and Buffy Coat sample</td>
<td>1 Box (11 Aliquots)</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>
vi. Tally the Total Price within the last column for all goods included in shipment and record appropriately.

1. Reminder: the total price/value of the shipment should not exceed $2,500.

vii. Complete the final section with your signature.

viii. Specimens should be sent to the below address via FedEx Priority Overnight. Ambient FedEx shipments should be sent Monday through Thursday. Frozen FedEx Shipments should only be sent Monday through Wednesday.

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

8.4 Clinical Labs Shipping

1. Notify the IU Pathology Lab of shipment by emailing IU Path Lab study contacts at: kcleary@IUHealth.org, PJordan@IUHealth.org, rball3@IUHEALTH.ORG, AND jminch1@iuhealth.org.

   a. Attach the following to the email:
      i. Completed IU Path Lab Requisition Form (Appendix D).

         1. Fill out the following on the form:
            a. ID in this format: last name = BDS (already printed on template)
            b. First name = the 7 numerals of the rest of the ABC-DS ID (e.g., 024007).
            c. Date of collection
            d. Male/Female
            e. DOB is required in the system to register the sample. You can use the participant’s true DOB or a generic DOB (e.g., 01/01/1950). Either way, the DOB on the req form has to match the DOB on the Site BDS ID and DOB Label.
            f. MRN: NCRAD will generate this

         ii. If email is unavailable please call IU Path Lab and do not ship until you’ve contacted and notified IU Path Lab study contacts about the shipment in advance.

         iii. Please include the tracking number in the body of the email.
2. Label the 25-slot cryobox with the Site BDS ID and DOB Label.
   a. **Important Note:** The DOB on the IU Path Lab Req form needs to match the DOB on the Site BDS ID and DOB Label.
3. Place all refrigerated, labeled aliquots of serum from the same subject in the cryobox.
   a. Each 25-slot cryobox will hold approximately 4 serum samples.
   b. Cryoboxes should contain all specimens from the same participant, per time point.
   c. Place 3mL EDTA tube in bubble wrap tube sleeve and place within the SAME biohazard bag as cryobox containing serum aliquots. These biohazard bags are large enough to contain one cryobox and the EDTA tube.
   d. 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.
   e. **Ensure fluorescent round sticker is on biohazard bag.**
4. Place biohazard bag within X-Small Insulated shipper with 2 cold brick packs.
5. Place X-Small Insulated shipper within brown corrugated box and include air pouches.

6. Place fluorescent rectangular sticker on outside of brown corrugated box.

7. Include original copy of the IU Path Lab Req Form (Appendix D).

8. Seal the outer cardboard shipping carton with packing tape.

9. Apply all provided warning labels and the UPS Next Day Air return airbill (pre-printed and included in the kit) on the outside of the package. Do not overlap labels.
   a. Ensure the large rectangular fluorescent sticker is on the outside of the brown corrugated box.
   b. Specimens should be sent to the below address via UPS Next Day Air. Refrigerated shipments should be sent Monday through Friday.

   **Important Note**: It is vital to properly notify the IU Path Lab team of sample shipment, especially when shipping on Fridays! The IU Path Lab building is locked on the weekend, therefore one of the staff members will have to let the delivery driver in to complete delivery. Ensure the IU Path Lab requisition form is properly completed and the tubes properly labeled to avoid verification issues and delayed results.

   c. Schedule a pick-up using the following link: Schedule a Pickup | UPS - United States. You will need to provide the tracking number found on the pre-printed airbill and UPS account number.

   **ABC-DS Study at IU Path Lab**
   IU Health Pathology Laboratory
   350 W. 11th Street
   5th Floor, Rm 5013
   Indianapolis, IN 46202
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.

The Alzheimer’s Therapeutic Research Institute (ATRI) 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 ATRI 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.

Results from karyotyping will be uploaded to the ABC-DS EDC site at ATRI by the NCRAD study coordinator 7-10 days after receipt into the laboratory. You can find the results in your site folder: Docs → Site Topics → Choose Site Folder. To set notifications so you know when a report has been uploaded, first go to the "Docs" tab, then click "Manage Notifications" to the right of the search bar. Select a notification for 'file added' or other choices shown.

Clinical lab results will be available through the IU Health Lifepoint application. To access site specific participant results, study personnel must complete an “Access Request – Lifepoint, IU Non-Employee Form” and submit directly to IU Health. Social Security Number can be documented as “n/a” if the form is signed off on by the Field Site Lead in place of Manager. IU Health will send log-in information to you directly. The ABC-DS Admin Core will not need copies of these set up documents; however, please inform us who from your site will be designated to access the Lifepoint portal.

The ‘group data’ for all participants will be sent from the IU Health Path Lab to LONI, for purposes of analysis. (Site and participant IDs will be removed and new ID assigned per ABC-DS protocol.)

*Please check the portal for results ASAP in case a test fails and a re-draw is in order. Saturday deliveries: If issues arise with the specimens, the IU Path Lab will perform the tests offline. The following Monday, after review and corrections, results will be posted.*

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 ATRI 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 Appendixes List

Appendix A: Rate of Centrifugation Worksheet

Appendix B: Biological Sample and Shipment Notification Form

Appendix C: International Commercial Invoice

Appendix D: IU Path Lab Req Form

Appendix E: Constitutional (Blood) Test Requisition Form

Appendix F: UNTHSC Intake Form

Appendix G: UNTHSC Import Batch 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
alzstudy@iu.edu
Appendix B: Biological Sample and Shipment Notification Form (link)

Appendix B

Sample Collection - Blood & Shipment Notification Form

PT ID: ______ Site ID: ______
Cycle Visit (Circle One): 1 2 3 4

General Information:
From: ____________________________
Phone: ____________________________
PT previously enrolled in (circle one): AIDS NIAD N/A-new PT
NIAD/NIADD Legacy ID (if applicable): ____________
Arm: [ ] DS Participant [ ] Sibling Control
Sex: [ ] M [ ] F Year of Birth: ____________
Shipment Tracking #: ____________________________

Field Draw?: [ ] Yes [ ] No

Kit #: ____________________________
Kit Barcode: ____________________________

Blood Collection:
1. Date Drawn: ____________ [YYYYMMDD]
2. Time of Draw (24 hour clock): ____________ [HHMM]

Last time subject ate (date): ____________ [YYYYMMDD]

Blood Processing:

| Original volume drawn (5 mL RNA Pakgene tube) | Time placed in freezer: [HHMM] |
| ln 12.5 mL RNA Pakgene tube | mL |
| 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 (10 mL Naïve tube): mL

Serum (Serum Separator/Gold Top Tube)

| Original volume drawn (215 mL Serum Tube): mL |
| Time spin started (24 hour clock): [HHMM] |
| Duration of centrifuge: [minutes] |
| Temp of centrifuge: °C | Rate of centrifuge: x g |

Number of 0.25 mL plasma aliquots created (25-40 total) 
(Siliconized cryovial): x 0.25 mL

Number of 0.25 mL plasma aliquots sent to UNTHSC: ______
(Siliconized cryovial): x 0.25 mL

Number of 0.25 mL serum aliquots created (10-20 total) (Siliconized cryovial):
(Siliconized cryovial): x 0.25 mL

Number of 0.25 mL serum aliquots sent to UNTHSC: ______
(Siliconized cryovial): x 0.25 mL

Notes:

Version 10.2021
Appendix C: International Commercial Invoice (link)

```
COMMERCIAL INVOICE

This invoice must be completed in English.

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<tr>
<td>Air Waybill/Tracking No.</td>
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<tr>
<td>Payment Terms</td>
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<td>Purpose of Shipment</td>
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<td>Contact Name</td>
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<td>Telephone No.</td>
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</tr>
<tr>
<td>Company Name/Address</td>
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<td>Company Name/Address</td>
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<td>Country/Territory</td>
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<tr>
<td>Parties to Transaction</td>
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<td>Non-Related</td>
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<td>Tax ID</td>
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<td>Contact Name</td>
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<tr>
<td>Telephone No.</td>
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<th>Field</th>
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<tr>
<td>Description of Goods</td>
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<tr>
<td>Harmonized Tariff Number</td>
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</tr>
<tr>
<td>Country of Manufacture</td>
<td></td>
</tr>
<tr>
<td>Unit Value</td>
<td></td>
</tr>
<tr>
<td>Total Value</td>
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<th>Field</th>
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<tbody>
<tr>
<td>Terms of Sale</td>
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<td>Special Instructions</td>
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<td>Declaration Statement(s)</td>
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<th>Field</th>
<th>Value</th>
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<tbody>
<tr>
<td>Signature/Title/Date</td>
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</table>

Rev. 01/01/20-1.08

Version (9.2022)
Appendix D: IU Path Lab Req Form

Indiana University Health

Study/Research Lab Orders

IU Health Pathology Laboratory
350 W. 11th Street, Rm 5013
Indianapolis, IN 46202
317.491.6000 or 800.433.0740
Fax: 317.491.6001

<table>
<thead>
<tr>
<th>Test Code</th>
<th>Test Name</th>
<th>Select Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>7598</td>
<td>1,25 Dihydroxyvitamin D</td>
<td>Cycle 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cycle 2</td>
</tr>
<tr>
<td>7432</td>
<td>Anti-Thyroglobulin Antibody QN</td>
<td>Cycle 1</td>
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<tr>
<td></td>
<td></td>
<td>Cycle 2</td>
</tr>
<tr>
<td>6917</td>
<td>Basic Metabolic Panel</td>
<td>Cycle 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cycle 2</td>
</tr>
<tr>
<td>127</td>
<td>CBC with Diff</td>
<td>Cycle 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cycle 2</td>
</tr>
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<td>6318</td>
<td>Hemoglobin A1C HPLC Bld QN</td>
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<tr>
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<td></td>
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<td>6039</td>
<td>Lipid Panel SerPl QN</td>
<td>Cycle 1</td>
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<tr>
<td></td>
<td></td>
<td>Cycle 2</td>
</tr>
<tr>
<td>6940</td>
<td>T4 Free Direct SerPl QN</td>
<td>Cycle 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cycle 2</td>
</tr>
<tr>
<td>7699</td>
<td>Thyroid Peroxidase Ab</td>
<td>Cycle 1</td>
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<tr>
<td></td>
<td></td>
<td>Cycle 2</td>
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<td>7430</td>
<td>Triiodothyronine Ser QN (T3 Total)</td>
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<tr>
<td></td>
<td></td>
<td>Cycle 2</td>
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<tr>
<td>7339</td>
<td>TSH 3rd Generation SerPl QN</td>
<td>Cycle 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cycle 2</td>
</tr>
<tr>
<td>6691</td>
<td>Vitamin B12 SerPl QN</td>
<td>Cycle 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cycle 2</td>
</tr>
</tbody>
</table>
Appendix F: UNTHSC Intake Form (link)

*Click link to view all pages*

**UNTHSC Sample Shipping Process**

We appreciate your time and dedication to this project; with that, we want to ensure the best scenario for your samples upon arrival and best possible test results.

Our testing is a highly automated process requiring a good deal of preparation prior to any testing. In order for the Institute for Translational Research Laboratory to be prepared for the upcoming shipment of your samples, we ask that you answer a few questions regarding your samples as this will prevent any delay in obtaining your results.

***MINIMUM VOLUME REQUIREMENT*** 500ul of sample for MSD and 500ul of sample for Quanterix - Please discuss this with our lab personnel.

Please be sure to include:

- An excel file with the 5 columns listed below:
  - Unique Sample ID (Each sample is uniquely identified)- required
  - Unique TubeID/Barcode- required
  - Visit # (unique timepoint for each sample in the study)- required for multiple visits
  - Date of Collection- if applicable
  - Notes for sample (i.e. hemolyzed etc)- if applicable

<table>
<thead>
<tr>
<th>Unique Sample ID</th>
<th>Unique Tube ID/Barcode</th>
<th>Visit Number</th>
<th>Date of Collection</th>
<th>Notes for Samples</th>
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- Indicate sample type(s) to be sent
  - □ Plasma
  - □ Serum
  - □ Other ___________________________

- Number of samples per sample type_________________

- Volume of each sample (please add notes for any low volume samples).
  - □ Please note, any sample we declare as unusable will be discarded.
### Appendix G: UNTHSC Import Batch Form

<table>
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<th>OutsideId</th>
<th>OutsideTubId</th>
<th>OutsideNotes</th>
<th>OutsideDate</th>
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