Alzheimer’s Disease Center
Fluid Biomarkers Study

Collection and Shipment Training
Training Overview: ADC FB

- Study Overview
- Kit Review
- Sample Collection and Processing
- Sample Shipping
- Sample Form
- NCRAD Website
- Common Nonconformance Issues
- Questions?
Globally Unique Identifier (GUID)

- The GUID is a subject ID that allows researchers to share data specific to a study participant, without exposing personally identifiable information.
- A GUID is made up of random alpha-numeric characters and does not include any PHI in the identifier.
Globally Unique Identifier (GUID)

1. Create an account: https://bricsguid.nia.nih.gov/portal/jsp/login.jsp
2. Once you have an account, go to the GUID Tool – Create GUID
3. To open the ‘Launch GUID Tool’ you will need to have Java installed on your device
4. When the GUID Tool is open, you will need all of the following information
   - Complete legal given (first)name of participant at birth
   - The participant’s middle name, if applicable
   - Complete legal family (last) name of subject at birth
   - Day of birth
   - Month of birth
   - Year of birth
   - Name of city/municipality in which subject was born
   - Country of birth
# ADCFB Kits

<table>
<thead>
<tr>
<th>Total Blood Volume Collected</th>
<th>20 ml</th>
<th>40 ml</th>
<th>50 ml</th>
<th>52.5 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kit Type</td>
<td>20</td>
<td>40</td>
<td>50</td>
<td>52</td>
</tr>
</tbody>
</table>
# ADC FB Study Specimens

<table>
<thead>
<tr>
<th>Biospecimen</th>
<th>All Visits</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC</td>
<td>X</td>
</tr>
<tr>
<td>Plasma</td>
<td>X</td>
</tr>
<tr>
<td>Buffy Coat (DNA)</td>
<td>X</td>
</tr>
<tr>
<td>Serum</td>
<td>X</td>
</tr>
<tr>
<td>Whole Blood (RNA)</td>
<td>X</td>
</tr>
</tbody>
</table>
Kit Request Module

http://kits.iu.edu/adcfb/
ADCFB Kit Request Module

- Choose your site from the drop-down list.
- The coordinator name and contact information will appear.
- Verify that this information is accurate, or correct it if necessary.
- Indicate the quantity needed of each kit
- Once selected, kit components of the chosen kit will appear at the bottom of the screen
- Click “Submit” to turn in your request.
- **Note: You can order more than one type of kit in a single kit request**
Specimen Labels
Four Label Types

Kit Number

Site ID:____
PTID:

0001234567
ADC FB
PBMC
Kit #: 300001

Collection Tube

ADC FB
Plasma
Kit: 300001

Cryovial
Kit Number Labels

Used to track patient samples and provide quality assurance – Will be placed on the following locations:

1. Blood Sample and Shipment Notification Forms
2. Cryoboxes that house aliquots during shipping
3. One extra label provided

Provided by NCRAD in the kits
Site and PTID Labels

- Subjects will be identified by their Site and PTID
- Sites will be responsible for handwriting this onto the provided labels
  - Must use Fine Point Sharpie Marker
  - Each site will receive 3 markers in initial kit supply
Collection Tube Labels

- Collection Tube labels have 4 components:
  - 10 digit specimen barcode
  - Study name
  - Specimen type
  - Kit number
Cryovial Labels

- Only one label to be placed on each cryovial
  - **Plasma**
    - From EDTA tube
  - **Buffy Coat**
    - From EDTA tube
  - **Serum**
    - From Serum tube
Blood Collection Tubes

Label 1: Site and PTID label
- Site ID:
- PTID:

Label 2: Collection Tube label
- 0001234567
- ADC FB
- PBMC
- Kit #: 300001

All collection tubes will have two labels:
- The handwritten Site and PTID label
- The collection tube label
Labeling Biologic Samples

- Label all collection and aliquot tubes *before* cooling, collecting, processing or freezing samples.
- Label only 1 subject’s tubes at a time to avoid mix-ups.
- Wrap the label around the tube *horizontally*. Label position is important for *all* tube types.
- Make sure the label is completely adhered by rolling between your fingers.
- Do NOT cover barcode on cryovial with label.
Collection Tube Labeling

PBMC Tube

Collection Tube Label

Site and PTID Label
Handling/Processing Study Specimens
# Blood Draw Order

<table>
<thead>
<tr>
<th>Tube Type</th>
<th>Number of Tubes Drawn</th>
<th>Tube Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sodium Heparin (Green-Top) Tube (10 ml)</td>
<td>x2</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>2. EDTA (Purple-Top) Tube (10 ml)</td>
<td>x2</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>3. Serum (Red-Top) Tube (10 ml)</td>
<td>x1</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>4. PAXgene™ Tube (2.5 ml)</td>
<td>x1</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>
# Cryovial Cap Colors

<table>
<thead>
<tr>
<th>Cap Color</th>
<th>Sample Type</th>
<th>Cap Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purple</td>
<td>Plasma</td>
<td><img src="image1.png" alt="Purple Cap Image" /></td>
</tr>
<tr>
<td>Gray</td>
<td>Buffy Coat</td>
<td><img src="image2.png" alt="Gray Cap Image" /></td>
</tr>
<tr>
<td>Red</td>
<td>Serum</td>
<td><img src="image3.png" alt="Red Cap Image" /></td>
</tr>
</tbody>
</table>
PBMC Collection

- 2 x Sodium heparin (green top) BD Vacutainer® (10 ml)
- Not processed at site
- *NOTE*: Must be shipped AMBIENT to NCRAD the day sample is drawn. No Friday Draws.
PBMC Preparation (10ml Sodium Heparin Tube x 2)

**Step One**
- Collect blood in Sodium Heparin tubes allowing blood to flow for 10 seconds, and ensuring blood flow has stopped.

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

**Step Three**
- Store tubes at room temp. until shipment.
- Ship ambient same day as blood draw.

**Step Four**
- Label tubes with pre-printed PTID and collection tube labels prior to blood draw.
Plasma Collection

Create up to 7 aliquots
(Six 1.5ml aliquots in purple caps + 1 residual aliquot in blue cap)
Buffy Coat Collection
**Plasma/Buffy Coat Collection and Processing**

**Step One**
Store tubes at room temp.
Each tube should be labeled with Collection Tube and Site and PTID Labels.

**Step Two**
Collect blood into each EDTA Tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

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

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

**Step Five**
Centrifuge samples at 2000 x g for 10 minutes at 4°C.

**Step Six**
Pool all plasma from the 2 EDTA tubes into a 15 ml conical tube and invert gently 3 times to mix the plasma.

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

**Step Eight**
- Label gray-capped cryovials with “BUFFY COAT” labels.
- Using a clean transfer pipette, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat from each EDTA tube into its own cryovial.
- Store buffy coat aliquots upright at -80°C until shipment to NCRAD.
- Spin, aliquot, and freeze all plasma and buffy coat aliquots within 1 hour of collection.
Serum Tube

** Please note: After standing at room temperature for 30 minutes, blood will be clotted and immobile**
Serum Preparation

Step One: Store tubes at room temp.
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 tube 5 times to mix sample.
Step Four: Allow blood to clot for 30 minutes.
Step Five: Within 45 minutes of blood draw, centrifuge samples at 2000 x g for 10 minutes at 4°C.

Label three red-capped cryovials and one blue-capped cryovial with “SERUM” labels. Alloquote 1.5 ml into each cryovial. If residual aliquot is created, document specimen number and volume on Sample Form. Store serum aliquots upright at -80°C until shipment. Spin, aliquot, and freeze aliquots within 1 hour of collection.
RNA PAXgene™ Tubes for RNA
RNA Preparation (2.5ml PAXgene™ Tube)

Step One
- Store tubes at room temp.
- Label tubes with pre-printed subject 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 tube 8-10 times to mix sample.

Step Four
- Store tubes in wire rack at -80°C until shipment to NCRAD.
- Do not store tube in Styrofoam racks.
Sample Shipments
Sample Shipment Summary

<table>
<thead>
<tr>
<th>Collection Tube</th>
<th>Drawn At</th>
<th>Specimen Type</th>
<th>Aliquot Volume</th>
<th>Total Number of Aliquots</th>
<th>Shipping Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Sodium Heparin (Green-Top) Blood Collection Tubes (10 ml)</td>
<td>All Visits</td>
<td>Whole Blood</td>
<td>N/A</td>
<td>N/A</td>
<td>Ambient</td>
</tr>
<tr>
<td>2 EDTA (Purple-Top) Blood Collection Tubes (10 ml)</td>
<td>All Visits</td>
<td>Plasma</td>
<td>1.5 ml plasma aliquots</td>
<td>Up to 7</td>
<td>Frozen</td>
</tr>
<tr>
<td></td>
<td>All Visits</td>
<td>Buffy Coat</td>
<td>~1.0 ml buffy coat aliquots</td>
<td>2</td>
<td>Frozen</td>
</tr>
<tr>
<td>1 Serum (Red-Top) Blood Collection Tubes (10 ml)</td>
<td>All Visits</td>
<td>Serum</td>
<td>1.5 ml serum aliquots</td>
<td>Up to 4</td>
<td>Frozen</td>
</tr>
<tr>
<td>1 PAXgene™ Blood Collection Tube (2.5 ml)</td>
<td>All Visits</td>
<td>Whole Blood</td>
<td>N/A</td>
<td>N/A</td>
<td>Frozen</td>
</tr>
</tbody>
</table>
Ambient Samples

- Two 10 ml PBMC samples
- Only Monday-Thursday collection and same day shipping. Plan ahead to schedule UPS.
- Samples must be received at NCRAD one day after collection.
- Do NOT draw or ship ambient samples on Friday
- Include copy of Blood Sample Shipment and Notification Form
Place the ambient PBMC tubes in the absorbent slots and biohazard bag.

Place the bag inside the small shipping box, and then set the refrigerant pack on top of it.

Place small shipping box within a provided UPS Laboratory Pak, seal, and place UPS label on outside of package.

*Gel packs must be put in a freezer at minimum the night before shipping.
Frozen Shipment Packaging

- All other samples shipped frozen to NCRAD
  - Plasma, Buffy Coat, Serum, PAXgene™
  - **Ship Monday-Wednesday Only**

- Hold packaged samples in a -80°C freezer until pickup

- Include copy of Blood Sample Shipment and Notification Form

- Batch samples together
  - 8 cryoboxes
  - **Batch shipping should be performed quarterly or as a full shipment of specimens accumulates, whichever is sooner.**
Shipping Frozen Samples

Plasma, Buffy Coat, and Serum Samples
Use the biohazard bag to package the 25-Slot cryobox and PAXgene™ tube (in the bubble slot)

Frozen Shipment Packaging

- Cryovial box placed in clear biohazard bag
- PAXgene™ tube in bubble slot
Frozen Shipment Packaging

- Place 2-3 inches of dry ice in the bottom of the Styrofoam shipping container, then insert the cryoboxes laying upright.

- Fully cover the cryoboxes with about 2 inches of dry ice in the provided shipper.

- Each Styrofoam shipper must contain about 45 lbs (20 kg) of dry ice.
Frozen Shipping – Dry Ice Requirements

Dry Ice label should not be covered with other stickers and must be completed or the shipping carrier will reject/return your package!

Net weight of dry ice in kg

20 kg of Dry Ice
Blood Sample and Shipment Notification Form

- A copy of the sample form *must* be emailed or faxed to NCRAD prior to the date of sample arrival.
- Please include sample forms in all shipments of frozen and ambient samples.
- Email: alzstudy@iu.edu
- Fax: 317-321-2003
<table>
<thead>
<tr>
<th>Blood Sample and Shipment Notification Form</th>
</tr>
</thead>
</table>
| **From:** Kelley Fisler  
**Email:** kmfisler@iu.edu  
**Phone:** 1-800-528-2539  
**Site ID:**  
**PT ID:**  
**GUID:**  
**NACC Visit:** |
| **Study:** ADC FB  
**Sex:** [ ] M  
**Year of Birth:**  
**Date of Draw:** [MMDDYY]  
**Time of Draw:** [HHMM]  
**Date subject last ate:** [MMDDYY]  
**Time subject last ate:** [HHMM] |
| **RBMC (NalAve Tube)**: N/A  
**RNA (PAXgene Tube)**: N/A |
| **#1**  
**Specimen Number (Last four digits):**  
**Original volume drawn:** ml  
| **#2**  
**Specimen Number (Last four digits):**  
**Original volume drawn:** ml |
| **Blood Processing**  
**EDTA #1 specimen number (Last four digits):**  
**Original blood volume of EDTA #1:** ml  
**Time spin started:** [HHMM]  
**Temp of centrifuge:** °C  
**Time aliquoted:** [HHMM]  
| **EDTA #2 specimen number (Last four digits):**  
**Original blood volume of EDTA #2:** ml  
**Duration of centrifuge:** min  
**Rate of centrifuge:** x g  
**Number of 1.5 ml plasma aliquots created:** (purple cap, up to 6) |
| **If applicable, volume of residual plasma aliquot (less than 1.5 ml in blue cap):** ml  
**Buffco #1 specimen number (Last four digits):**  
** Buffco #1 volume:** ml  
| **If applicable, specimen number of residual plasma aliquot (last four digits):**  
**Buffco #2 specimen number (Last four digits):**  
**Buffco #2 volume:** ml  
| **Time aliquots placed in freezer:** [HHMM]  
**Storage temperature of freezer:** °C |
| **Serum (Serum Tube)**: N/A  
**Time spin started:** [HHMM]  
**Temp of centrifuge:** °C  
| **Duration of centrifuge:** min  
**Rate of centrifuge:** x g  
| **Time aliquoted:** [HHMM]  
**Number of 1.5 ml serum aliquots created:** (red cap, up to 3)  
| **If applicable, volume of residual serum aliquot (less than 1.5 ml in blue cap):** ml  
**Time aliquots placed in freezer:** [HHMM]  
**Storage temperature of freezer:** °C |
| **Notes:** |
NCRAD Website: Helpful Pages

https://ncrad.org/holiday_closures.html
https://ncrad.org/friday_blood_draws.html

### Holiday Closures

<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.</td>
</tr>
<tr>
<td>4th Monday in May</td>
<td>Memorial Day</td>
</tr>
<tr>
<td>July 4</td>
<td>Independence Day (observed)</td>
</tr>
<tr>
<td>1st Monday in September</td>
<td>Labor Day</td>
</tr>
<tr>
<td>4th Thursday in November</td>
<td>Thanksgiving</td>
</tr>
<tr>
<td>4th Friday in November</td>
<td>Friday after Thanksgiving</td>
</tr>
<tr>
<td>December 25</td>
<td>Christmas</td>
</tr>
</tbody>
</table>

### What to do for Friday Blood Draws

NCRAD is not open for business on Saturday or Sunday; therefore, we ask that no samples be shipped on a Friday. We cannot guarantee the conditions in which the samples will be held by the shipping courier over the weekend. It is important to have plans in place for each type of sample to be held over the weekend prior to shipping. Please refer to the table below for how to handle samples drawn on a Friday.

When possible, please only ship frozen samples on Monday-Wednesday. There is always the potential for an unexpected shipping courier delay and by shipping Monday through Wednesday there should be enough time to receive the samples before the weekend.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Tube Type</th>
<th>Product</th>
<th>Shipment Method</th>
<th>Friady Draw Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>Sodium Heparin</td>
<td>PBMC</td>
<td>Ambient</td>
<td>DO NOT DRAW ON FRIDAY. Must be drawn on Monday - Thursday.</td>
</tr>
</tbody>
</table>

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NCRAD
Nonconformance Issues

Sample aliquots and collection tubes frozen at an angle/inverted

Recommendation: Place aliquots in Argos boxes/tube rack in freezer upright until shipment

Fields left blank on Blood Sample and Shipment Notification Form

Recommendation: Complete Sample Notification forms during the participant study visit as samples are processed.

Last time subject ate often left blank/unknown

Incorrect data reported on Sample and Shipment Notification Forms
Nonconformance Issues

Multiple low volume aliquots

Recommendation:
Lay out cryovials in a row and aliquot in order until sample is depleted

1.5 ml

YES

NO
Nonconformance Issues

- All frozen samples for a participant not sent within one shipment box (plasma, buffy coat, and serum aliquots should be kept together)

  Recommendation: Ship Samples to NCRAD utilizing the Notification Form, by PTID. Do not throw away labels until samples are packed and shipped.

- Aliquots arriving to NCRAD without labels

- Sample forms not faxed or scanned to NCRAD the day before shipment

- Ambient PBMC samples not shipped to NCRAD the day of blood draw

  Recommendation: No samples should be held ambient for any period of time at the site. Ensure all ambient PBMC samples are shipped by end of the day.
Contact Information

Kaci Lacy
- Phone: (317) 278-1170
- E-mail: lacy@iu.edu

General NCRAD Contact
- Phone: (800) 526-2839
- E-mail: alzstudy@iu.edu
- Website: www.ncrad.org