Alzheimer’s Disease in Down Syndrome (ADDS)

in collaboration with

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

Blood-Based Biospecimen Training Slides
Contact Information

• Questions?
  Please contact NCRAD Coordinators at:
  • Phone: 1-800-526-2839
  • E-mail: alzstudy@iu.edu
  • Website: www.ncrad.org
Training Overview:

- Blood-Based Collection Schedule
- Kit Request Module
- Specimen Labels
- Handling/Processing Study Specimens
- Sample Shipping
- NCRAD Website
- Questions?
### ADDS Blood Based Collection Schedule:

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

http://kits.iu.edu/adds-niad
Kit Request Module

• An initial stock of kits will be delivered prior to the designated site specific start date.

• Kits and individual supplies are available to order:
  • ADDS Blood Kit
  • ADDS Frozen Blood Shipping Supply Kit
  • Blood Supplemental Kit
  • CSF Supplemental Supply Kit
  • Lumbar Puncture Trays
  • Frozen CSF Shipping Supply Kit
NCRAD Kit Request Module

1. Choose your site from the drop down list
2. The coordinator name and contact information will appear
3. Verify that this information is accurate, correct if necessary
ADDS and NiAD Kits Available

<table>
<thead>
<tr>
<th>NiAD Sibling Control Blood Kit Qty</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NiAD DS Participant Blood Kit Qty</td>
<td></td>
</tr>
<tr>
<td>ADDS Blood Kit Qty</td>
<td></td>
</tr>
<tr>
<td>NiAD Frozen Blood Shipping Kit Qty</td>
<td></td>
</tr>
<tr>
<td>ADDS Frozen Blood Shipping Kit Qty</td>
<td></td>
</tr>
<tr>
<td>Blood Supplemental Supply Kit Qty</td>
<td></td>
</tr>
<tr>
<td>CSF Supplemental Supply Kit Qty</td>
<td></td>
</tr>
<tr>
<td>Lumbar Puncture Tray Kit Qty</td>
<td></td>
</tr>
<tr>
<td>Frozen CSF Shipping Supply Kit Qty</td>
<td></td>
</tr>
</tbody>
</table>

**Do you need Extra Supplies?**
1. [ ] Yes
2. [ ] No

**Comments**

[Submit]
**Study Visit Kits**

- Indicate the quantity needed of each kit
- Once selected, kit components of the chosen kit will appear at the bottom of the screen (Pictured)
- Click “Submit” to turn in your request.
- The IU staff will notify you that your request has been received and address any issues.
- **Note: You can order more than one type of kit in a single kit request**

<table>
<thead>
<tr>
<th>Study Visit Kit</th>
<th>Qty</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIAD Sibling Control Blood Kit Qty</td>
<td></td>
</tr>
<tr>
<td>NIAD DS Participant Blood Kit Qty</td>
<td></td>
</tr>
<tr>
<td>ADDS Blood Kit Qty</td>
<td>1</td>
</tr>
<tr>
<td>NIAD Frozen Blood Shipping Kit Qty</td>
<td></td>
</tr>
<tr>
<td>ADDS Frozen Blood Shipping Kit Qty</td>
<td></td>
</tr>
<tr>
<td>Blood Supplemental Supply Kit Qty</td>
<td></td>
</tr>
<tr>
<td>CSF Supplemental Supply Kit Qty</td>
<td></td>
</tr>
<tr>
<td>Lumbar Puncture Tray Kit Qty</td>
<td></td>
</tr>
<tr>
<td>Frozen CSF Shipping Supply Kit Qty</td>
<td></td>
</tr>
</tbody>
</table>

**Do you need Extra Supplies?**
- Yes
- No

**Comments**

Each ADDS Blood Kit Contains:
1. EDTA (Lavender-Top) Blood Collection Tube (10 ml)
2. Serum Separator (Gold-Top) Blood Collection Tube (5 ml)
3. Siliconized cryovial tube (0.5 ml) with lavender sticker
4. Siliconized cryovial tube (0.5 ml) with red sticker
5. 15 ml conical
6. Cryovial tube (2.0 ml) with blue cap
7. Disposable graduated transfer pipette
8. Pre-printed Collection and Aliquot Tube Label
9. Pre-printed Kit Number Label
10. Labels for handwritten Site and Subject ID
11. Microcentrifuge tube box (holds up to 25 microcuvorials)

[Submit button]
Specimen Labels
Label Type Summary

1. Kit Number Labels
2. Site and Subject ID Labels
3. Collection and Aliquot Tube Labels
   • Differ by specimen type
Kit Number Labels

- Used to track patient samples and provide quality assurance
- Will be placed on the following locations:
  1. Biological Sample and Shipment Notification Form
  2. Outside cryobox that houses aliquot tubes during storage and shipment

Provided by NCRAD in the kits
Site and Subject ID Label

- Subjects will be identified by their site ID and subject ID
- The subject ID may only be available shortly before the visit
- Sites will be responsible for handwriting this onto the provided labels
  - Must use fine point permanent marker
  - Each site will receive 4 markers in initial kit supply
Site and Subject ID Label Cont.

- Write information on label prior to adhering to tube
- Label will be placed on all collection tubes
  - (2) Serum Separator (Gold-Top) Blood Collection Tube (5 ml)
  - EDTA (Lavender-Top) Blood Collection Tube (10 ml)
- Kits will include one extra label
Collection and Aliquot Tube Labels

Specimen Number (assigned by NCRAD)

Repository Name

Sample Type

Kit # (assigned by NCRAD) unique to the subject and visit
Collection and Aliquot Tube Labels - Blood

- Labels to be placed on ALL collection and aliquot tubes

1. Serum Separator (Gold-Top) Blood Collection Tube (x2)
   - Serum aliquots
2. EDTA (Lavender-Top) Blood Collection Tube (x1)
   - Plasma aliquots
   - Buffy coat aliquot
Collection Tubes – Blood

- Serum Separator (Gold-Top) Blood Collection Tube (5 ml)
- EDTA (Lavender-Top) Blood Collection Tube (10 ml)

Collection/Aliquot tube label
*place barcode near top

Site and Subject ID Label
Collection Tubes - Blood

Label 1: Collection Tube Label

- EDTA Tube
- Serum Separator Tubes

Label 2: Site and ID Label

- Site: _____
- ID: __________________

- All collection tubes will have two labels
  - The Collection Tube Labels
  - The handwritten Site and Subject ID Label
Aliquot Tube Labels – Serum, Plasma and Buffy Coat

- Collection and Aliquot tube label only
- Please place barcode near cap
Each site will be responsible for ordering kits (labels included) and maintaining a supplies on site for scheduled participants.

To order, sites will use the Indiana University online kit ordering module: [http://kits.iu.edu/adds-niad](http://kits.iu.edu/adds-niad)

Allow a minimum of **2 weeks** for your order to be processed and delivered.
Handling/Processing Study Specimens
# Site Required Equipment

## Blood Collection/Safety Equipment

1. **PPE**
   - Lab Coat, Safety Glasses
2. Tourniquet
3. Alcohol Prep Pad
4. Gauze Pad
5. Butterfly Needles
6. Bandage
7. Sharps Bin and Lid

## Processing/Storage Equipment

1. Centrifuge capable of $\geq 2000$ rcf with refrigeration to $4^\circ\text{C}$
2. -80°C Freezer
3. Wet Ice Bucket
***Important Note***

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

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

<table>
<thead>
<tr>
<th>Tube Type</th>
<th>Number of Tubes Drawn</th>
<th>Tube Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Serum Separator (Gold-Top) Blood Collection Tube (5 ml)</td>
<td>x2</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>2. EDTA (Lavender-Top) Blood Collection Tube (10 ml)</td>
<td>x1</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
</tbody>
</table>
## Aliquot Cap Colors

<table>
<thead>
<tr>
<th>Cap Color</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear Cap with Lavendar Sticker</td>
<td>Plasma and Plasma Residual (&lt;0.25 ml) (Document Specimen Number of Residual Aliquot on Sample Form)</td>
</tr>
<tr>
<td>Clear Cap with Red Sticker</td>
<td>Serum and Serum Residual (&lt;0.25 ml) (Document Specimen Number of Residual Aliquot on Sample Form)</td>
</tr>
<tr>
<td>Blue</td>
<td>Buffy Coat</td>
</tr>
</tbody>
</table>
Serum Preparation (5 ml Gold-Top Tube)

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

Step Two
- Collect blood in (2) 5 mL Gold-Top tubes allowing blood to flow for 10 seconds and ensure blood flow has stopped.

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

Step Four
- Allow blood to clot for 30 minutes.
- Within 60 minutes of blood draw, centrifuge samples at 2000 x g at 4°C for 10 minutes
- Using a clean transfer pipette, transfer Serum from both 5 mL Gold-Top tubes to the 15 mL conical tube.
- Mix the 15 mL conical tube gently by inverting 3-4 times.

Step Five
- Adhere preprinted labels to the clear cap cryovials with red stickers
- Aliquot 0.25 ml into each cryovial tube.
- If a residual aliquot is created, document specimen number and volume on Sample Notification Form.
- Store serum aliquots at -80°C until shipment.
Serum Separator Tube (Serum Collection)

Immediately after Blood Draw

Create 16 to 21 aliquots of 0.25ml; if residual aliquot created, document specimen number and volume on sample form
Plasma and Buffy Coat Preparation (10ml Lavender-Top Tube)

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

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

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

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

**Step Five**
- Preferably within 30 minutes of blood draw, centrifuge samples at 2000 x g for 10 minutes.
- Samples need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.

**Step Six**
- Adhere preprinted labels to the clear cap cryovials with lavender stickers.
- Aliquot 0.25 ml into each cryovial tube.
- Store plasma aliquots at -80°C until shipment.

**Step Seven**
- Adhere preprinted labels to the blue cap cryovial.
- Using a clean pipette tip, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat into the cryovial tube.
- Store buffy coat aliquot at -80°C until shipment.
Create 16 to 21 aliquots of 0.25ml; if residual aliquot created, document specimen number and volume on sample form
EDTA Tube (Buffy Coat Collection)

Important Note:
- Buffy Coat aliquots will be distinguished from the plasma aliquots through a blue cap.
Sample Shipping
# Blood Sample Shipment Summary

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

• Ship Monday-Wednesday Only
  • Serum, Plasma and Buffy Coat

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

• Batch Samples together
  • 10 cryoboxes (5 participant biospecimens visits per shipment)
  • Batch shipping should be performed quarterly or as a full shipment of specimens accumulates, whichever is sooner.
Frozen Shipping – Cryoboxes

Place kit number label on top of cryobox

One cryobox to contain Serum aliquots, one per subject.

One cryobox to contain Plasma and Buffy Coat aliquots, one per subject.

Place both cryoboxes into one Biohazard Bag.
Shipping Frozen Samples

• Schedule FedEx

• *Send Biological Sample and Shipment Notification Form to IU (US) ahead of shipment*

  • Email: alzstudy@iu.edu or
  • Fax: 317-321-2003
Frozen Shipping – Dry Ice Requirements

- 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

Class 9 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

Your name & address

Repository name & address

Dry Ice kg

UN 1845

Airwaybills / airbills must have the following:
1. Dry ice; 9; UN 1845
2. (Number of packages) x (weight in kg)
Frozen Shipping - Fedex Airbill

Airbill must be completed or the shipping carrier will reject/return your package!

Your name, address & phone

Dangerous goods info (for dry ice shipments only)

Net weight of dry ice in kg

FedEx Account Number; will be prefilled for ADDS study
Biological Sample and Shipment Notification Forms

• 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
Send by E-mail or Fax prior to shipment, and include a copy in each shipment.

<table>
<thead>
<tr>
<th>Biological Sample Notification Form - Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood collected for:</strong></td>
</tr>
<tr>
<td>• Serum</td>
</tr>
<tr>
<td>• Plasma</td>
</tr>
<tr>
<td>• Buffy Coat</td>
</tr>
</tbody>
</table>

**To:** Kelley Faber  
**Email:** alzbstudy@ih.u.edu  
**FAX:** 317-321-2001  
**Phone:** 1-800-526-2839

**Subject ID:**  
**Site ID:**  
**MGH InitID:**  
**Cycle Visit:** 1A 1B 2A 2B 3A 3B

---

<table>
<thead>
<tr>
<th>Biological Sample and Shipment Notification Form</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General Information:</strong></td>
</tr>
<tr>
<td>From: _________________________________________</td>
</tr>
<tr>
<td>Phone: _____________________________</td>
</tr>
</tbody>
</table>
| Study: ADDS  
  DS Participant  
  Sex: M  
  Year of Birth: ____________  |
| Kit #: ________________________________________  |

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

| **Blood Processing:**                           |
| 
  Plasma (EDTA/Lavender Top Tube)                | Serum (Serum Separator/Gold Top Tube)         |
| • Time spin started (24 hour clock): [HHMM]     | • Time spin started (24 hour clock): [HHMM]    |
| • Duration of centrifuge: [minutes]             | • Duration of centrifuge: [minutes]            |
| • Temp of centrifuge: °C  
  • Rate of centrifuge: g x g                   | • Temp of centrifuge: °C  
  • Rate of centrifuge: g x g                   |
| Original volume drawn (1x50 mL EDTA tube):      | Original volume drawn (2x5 mL Serum tube):     |
| • Time aliquoted: [HHMM]                        | • Time aliquoted: [HHMM]                       |
| Number of 0.25 mL plasma aliquots created (16-20 total) (Simplified aliquot): | Number of 0.25 mL serum aliquots created (16-20 total) (Simplified aliquot): |

| • If applicable, volume of residual plasma aliquot [less than 0.25 mL] | • If applicable, volume of residual serum aliquot [less than 0.25 mL] |
| [ ] [ ] [ ] [ ]                                                      | [ ] [ ] [ ] [ ] |
| • If applicable, specimen number of residual aliquot (last four digits): | • If applicable, specimen number of residual aliquot (last four digits): |
| [ ] [ ] [ ] [ ]                                                      | [ ] [ ] [ ] [ ] |
| • Time aliquots placed in freezer (24 hour clock): [HHMM]             | • Time aliquots placed in freezer (24 hour clock): [HHMM] |
| • Storage temperature of freezer: °C                                 | • Storage temperature of freezer: °C |
| Buffy coat aliquot created [one per EDTA tube] (Blue cap cryovial):   | Note: Bulleted items not entered into eCRF. |
| [ ] [ ] [ ] [ ]                                                      | |

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**Version 03.2020**
NCRAD Website
Helpful Pages

- https://ncrad.org/holiday_closures.html
- https://ncrad.org/friday_blood_drews.html

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>Friday 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>
<tr>
<td>Whole Blood</td>
<td>EDTA Tube</td>
<td>DNA Only</td>
<td>Ambient</td>
<td>Do NOT refrigerate. Please keep sample at room temperature until the specimen can be shipped via next day delivery methods the following Monday.</td>
</tr>
</tbody>
</table>

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

https://www.ncrad.org/resource_adds_niad.html
ADDS/NiAD Active Study Page

Welcome ADDS/NiAD Study staff, coordinators, and PIs. This section encompasses study specific tools and videos for your reference. If you have any questions, comments, or new ideas, please contact NCRAD by email or phone 317-276-7505 or 317-276-2839.

ADDS Blood-Based Biomarker Collection Schedule for NCRAD:

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>3-Month</th>
<th>16-Month</th>
<th>19-Month</th>
<th>32-Month</th>
<th>33-Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td></td>
<td>✔️</td>
<td>✔️</td>
<td></td>
<td>✔️</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NIAD Blood-Based Biomarker Collection Schedule:

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>18-Month</th>
<th>22-Month</th>
<th>48-Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Plasma</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Serum</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>

* DNA from the 16-Month Visit (University of Pittsburgh Only) will not be shipped to NCRAD but maintained at the site.
** No Blood Based Biomarkers are collected at the 48-month visit.

NIAD Blood-Based Biomarker Collection Schedule for Sibling Controls:

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>16-Month</th>
<th>32-Month</th>
<th>48-Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>✔️</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Study Resources

- Kit Request Module
- Study Specific Sample Notification Forms
- ADDS/NiAD Manual of Procedures
- Study Related Video Tutorials
- ADDS/NiAD Training Slides
Contact Information

• Questions?

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