HEAD-to-head Evaluation of tau tracers in Alzheimer’s Disease

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: 317-274-7890
• CRC email: dlkeys@iupui.edu
• Back-up email: 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?
# HEAD Biospecimen Collection Schedule

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Baseline Visit</th>
<th>18 Month Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole Blood for RNA</strong></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Serum</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Plasma</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DNA</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Whole Blood for Banking</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**2.5 ml PAXgene™ Blood RNA Tube**
Kit Request Module

https://kits.iu.edu/head
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:
  • Blood-Based Kit
  • Frozen Blood Shipping Supply Kit
  • Blood-Based Supplemental Supply Kit (1 only at study start up)
  • Individual Supplies
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

Reminder: Please allow two weeks for kit orders to be processed and shipped

Is the contact name above correct?
- Yes
- No
### Kits Available

<table>
<thead>
<tr>
<th>HEAD Blood Collection Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Number of Blood Collection Kits Requested</strong></td>
</tr>
<tr>
<td>(same collection for BL, month 18)</td>
</tr>
<tr>
<td><strong>Total Number of Blood Supplemental Kits Requested</strong></td>
</tr>
<tr>
<td><em>Typically one per site per year</em></td>
</tr>
<tr>
<td><strong>Total Number of Frozen Blood Shipping Kits Requested</strong></td>
</tr>
<tr>
<td><em>Includes small frozen shipper</em></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HEAD Frozen Shipping Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Number of Frozen Shipping Kits Requested</strong></td>
</tr>
<tr>
<td><em>For batch shipments of 5-6 cryoboxes</em></td>
</tr>
</tbody>
</table>
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**
NCRAD Kit Request Module: When It Must be Used

• Each site will be responsible for ordering kits (labels included) and maintaining supplies on site for scheduled participants

• To order, sites will use the Indiana University online kit ordering module: https://kits.iu.edu/head

• Allow a minimum of **2-3 weeks** for your order to be processed and delivered.
Specimen Labels
Label Type Summary

1. Kit Number Labels
2. HEAD ID Labels
3. Collection Tube Labels
   • Differ by specimen type
4. Cryovial Labels
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
HEAD ID Label

- Subjects will be identified by their HEAD ID
- The HEAD 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
Collection Tube Labels

0001234567

HEAD

Plasma

Kit #: 300001

Specimen Number (assigned by NCRAD)

Study Name

Sample Type

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

Label 1: Collection Tube Label

- 0001234567
- HEAD
- Plasma
- Kit #: 300001

- 0001234567
- HEAD
- Buffy Coat
- Kit #: 300001

Collection/Aliquot tube label
*place barcode near top

HEAD ID label

Label 2: HEAD ID Label

HEAD:

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

- Cryovial tube label only
- Please don’t cover barcode on tube with label!
Labeling Biologic Samples

Please...

• 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.
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 ≥2000 rcf with refrigeration to 4°C
2. -80°C Freezer
3. Wet Ice Bucket
# Sample Collection - Blood

<table>
<thead>
<tr>
<th>Draw Tube Order</th>
<th>Tube Type</th>
<th>Number of Tubes Drawn</th>
<th>Tube Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PAXgene™ Tube for RNA</td>
<td>1</td>
<td><img src="image1.png" alt="Tube Image" /></td>
</tr>
<tr>
<td>2</td>
<td>Serum (Red-Top) Tube (10 ml)</td>
<td>1</td>
<td><img src="image2.png" alt="Tube Image" /></td>
</tr>
<tr>
<td>3</td>
<td>EDTA (Lavender-Top) Tube (10ml)</td>
<td>5</td>
<td><img src="image3.png" alt="Tube Image" /></td>
</tr>
<tr>
<td>4</td>
<td>EDTA (Lavender-Top) Tube (6 ml)</td>
<td>1</td>
<td><img src="image4.png" alt="Tube Image" /></td>
</tr>
</tbody>
</table>
## Aliquot Cap Colors

<table>
<thead>
<tr>
<th>Cap Color</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Cap</td>
<td>Serum</td>
</tr>
<tr>
<td>Purple Cap</td>
<td>Plasma</td>
</tr>
<tr>
<td>Blue Cap</td>
<td>Plasma Residual (&lt;1.5ml)</td>
</tr>
<tr>
<td></td>
<td>(Document Specimen Number and Volume of Residual Aliquot on Sample Form)</td>
</tr>
<tr>
<td>Gray Cap</td>
<td>Buffy Coat</td>
</tr>
</tbody>
</table>

![Image showing cap colors with labels]
RNA Preparation (2.5ml PAXgene™ Tube)

**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.
Serum Preparation (10ml Red Top Tube)

Step One
- Store tubes at room temperature.
- Label tubes and cryovials 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 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 for 10 minutes at 4°C.

Step Five
- Must be spun, aliquoted, and stored in -80°C freezer within 2 hours of collection.
- Adhere preprinted labels to the red-cap cryovials.
- Aliquot 1.5 ml into each cryovial tube.
- If a residual aliquot is created, document specimen number and volume on Sample Notification Form.
- Store serum aliquots at -80°C until shipment.
Serum Tube (Serum Collection)

** Please note: After standing at room temperature for 30 minutes, blood will be clotted and immobile**
Plasma and Buffy Coat Preparation (10ml Lavender-Top Tube x 5)

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

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

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

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

Step Five
- Centrifuge samples at 2000 x g at 4°C for 10 minutes.
- Samples need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.

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

Step Seven
- Adhere preprinted labels to the purple cap cryovials.
- Aliquot 1.5 ml into each cryovial tube.
- If a residual aliquot is created, document specimen number and volume on Sample Notification Form.
- Store plasma aliquots at -80°C until shipment.

Step Eight
- Adhere preprinted labels to the gray cap cryovials.
- Using a clean pipette tip, collect the Buffy coats (may have residual plasma and some RBCs included).
- Transfer the Buffy coats into the cryovial tubes.
- Store Buffy coat aliquots at -80°C until shipment.
EDTA Tube (Plasma Collection)

Plasma Aliquots (up to 17 possible)

Close up view of 2.0 ml cryovial
EDTA Tube (Buffy Coat Collection)

Important Note:
❖ Buffy Coat aliquots will be distinguished from the plasma aliquots through a gray cap.
Whole Blood Preparation (6 mL Lavender-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 tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three
- Immediately after blood draw, invert tube 3 times to mix sample.

Step Four
- Immediately after inversion, freeze the sample in an -80°C freezer until ready to ship.

Styrofoam racks
Sample Shipping
## Blood Sample Shipment Summary

<table>
<thead>
<tr>
<th>Draw Tube Order</th>
<th>Sample Type</th>
<th>Tube Type</th>
<th>Tubes to NCRAD</th>
<th>Ship</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Whole blood for transcriptome analysis</td>
<td>PAXgene™ Blood RNA Collection Tube (2.5 ml) for RNA</td>
<td>1</td>
<td>Frozen</td>
</tr>
<tr>
<td>2</td>
<td>Whole blood for isolation for serum</td>
<td>SERUM: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)</td>
<td>Up to 4</td>
<td>Frozen</td>
</tr>
<tr>
<td>3</td>
<td>Whole blood for isolation of plasma &amp; buffy coat (for DNA extraction)</td>
<td>PLASMA: 2.0 ml cryovials with purple cap (residual volume placed in 2.0 ml cryovial with blue cap)</td>
<td>Up to 17</td>
<td>Frozen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BUFFY COAT: 2.0 ml cryovial</td>
<td>5</td>
<td>Frozen</td>
</tr>
<tr>
<td>4</td>
<td>Whole blood for future analysis</td>
<td>EDTA (Lavender-Top) Blood Collection Tube (6 ml)</td>
<td>1</td>
<td>Frozen</td>
</tr>
</tbody>
</table>
Frozen Sample Shipping

• Ship Monday-Wednesday Only
  • Plasma
  • Buffy Coat
  • Serum
  • Whole Blood for RNA

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

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

Place frozen RNA (and frozen EDTA (6ml) tube, when applicable) in bubble wrap tube sleeves.

+ 

Place cryobox in one Biohazard Bag.
Shipping Frozen Samples

• Schedule UPS

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

  • *Email: alzstudy@iu.edu*
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

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

Contains 20.4 kg of Dry Ice
UPS ShipExec System

• Log into the ShipExec Thin Client at https://kits.iu.edu/ups
  • If a new user or contact needs access, please reach out to your study contact for access

• Follow instructions on section 8.2 of HEAD Manual of Procedures to create your return airbill and schedule a UPS pickup
Shipping Regulations and Training

PLEASE NOTE:

- All study personnel responsible for shipping should be certified in biospecimen shipping.
- It is the responsibility of each site to ensure that the appropriate training has been provided and conducted in regards to IATA shipping.
UN3373 Biological Substance, Category B Training

• Biological Substance, Category B are specimens being transported for “investigational purposes”
• Recommend: investigator sites document training of category B/dangerous goods
• We recommend establishing a record of your staff’s training and date of instruction
• The training records must be made available upon request by the appropriate national authority
  • Additional information from the Department of Transportation (DOT) can be found on their website http://hazmat.dot.gov
Biological Sample and Shipment Notification Forms

• A copy of the sample form *must* be emailed 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
Biological Sample Notification Form - Blood

**Send by E-mail and include a copy in each shipment**

<table>
<thead>
<tr>
<th>Blood collected for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Serum</td>
</tr>
<tr>
<td>• Plasma</td>
</tr>
<tr>
<td>• Buffy Coat/DNA</td>
</tr>
<tr>
<td>• Whole blood banking</td>
</tr>
<tr>
<td>• Whole Blood for RNA</td>
</tr>
</tbody>
</table>

---

### Blood Collection

- **Study:** HEAD
- **Visit (circle one):** BASELINE 18M

**Site ID:**

**PTID:**

**Sex (circle one):** M F

**Year of Birth:**

**Date Drawn:**

**Time of Draw:**

**Last date subject ate:**

**Last time subject ate:**

---

### Lavender Top Whole Blood Tube (6 ml)

<table>
<thead>
<tr>
<th>Original volume drawn</th>
<th>Time EDTA tube placed in freezer:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>RNA (PAXgene™ Tube)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original volume drawn</td>
</tr>
</tbody>
</table>

### Blood Processing:

**Serum (Red-top) Tube (10 ml):**

- **Time spin started:**
- **Number of 1.5 ml serum aliquots created (red cap):**

- **Duration of centrifuge:** Minutes
- **Temp of Centrifuge:** °C
- **Rate of centrifuge:** x g

**Plasma & Buffy Coat (Lavender-top) Tube (10 ml):**

- **Time spin started:**
- **Number of 1.5 ml plasma aliquots created (purple cap):** x 1.5 ml

- **Duration of centrifuge:** Minutes
- **Temp of Centrifuge:** °C
- **Rate of centrifuge:** x g

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**NOTES:**
NCRAD Website
Helpful Pages

• https://ncrad.org/holiday_closures.html
• https://ncrad.org/friday_blood_draws.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>Plasma</td>
<td>Polypropylene Aliquot Tubes</td>
<td>Plasma</td>
<td>Frozen</td>
<td>Plasma must be processed and aliquoted locally the day of collection. Once aliquoted, samples are stored upright in a -80°C freezer before shipment. The aliquots may then be packed with dry ice pellets and shipped frozen as the study MOP dictates.</td>
</tr>
<tr>
<td>Buffy Coat</td>
<td>Polypropylene Aliquot Tubes</td>
<td>DNA</td>
<td>Frozen</td>
<td>Buffy Coat aliquots must be processed and aliquoted locally the day of collection. Once aliquoted, the sample must then be transferred into a -80°C freezer until shipment. The aliquot may then be packed with dry ice pellets and shipped frozen as the study MOP dictates.</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>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</td>
</tr>
</tbody>
</table>
Welcome HEAD Study staff, coordinators, and PI's.
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 (800) 526-2839 or directly at (317) 278-1170.

### HEAD Biospecimen Collection Schedule

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>18 Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Plasma</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>DNA</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Whole Blood for banking</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Whole Blood for RNA**</td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>

**2.5 ml PAXgene™ RNA Tube for Whole Blood.

### Additional Resources
- Kit Request System
- Friday Blood Draws
- Shipping Address
- Holiday Closures

### Questions/Comments
- **Email:** alzstudy@iu.edu
- **Phone:** 800-526-2839
Contact Information

• Questions?

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