Bioimaging Fluid Biomarker Core

Collection and Shipment Training

NCRAD
Training Overview: BIFB

• Study Overview
• Kit Review
• Sample Collection and Processing
• Sample Shipping
• Sample Form
• NCRAD Website
• Common Nonconformance Issues
• Questions?
## BIFB Study Specimens

<table>
<thead>
<tr>
<th>Biospecimen</th>
<th>Baseline Visit</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>Whole Blood</td>
<td>X</td>
</tr>
<tr>
<td>Serum</td>
<td>X</td>
</tr>
<tr>
<td>CSF</td>
<td>X</td>
</tr>
</tbody>
</table>
Kit Request Module

http://kits.iu.edu/bifb/
NCRAD 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.
### BIFB Kits

| Specimen Collection Kit |  |
|-------------------------|  |
| BI-FB Collection Kit    |  |
| BI-FB LP Kit            |  |
| BI-FB CSF Kit           |  |

| Shipping Kits           |  |
|-------------------------|  |
| NCRAD Ambient Shipping Kit |  |
| NCRAD Frozen Shipping Kit (holds 8 subjects) |  |

| Supplemental Kits        |  |
|--------------------------|  |
| BI-FB Supplemental Supply Kit |  |
| BI-FB CSF Supplemental Supply Kit |  |

| Extra Supplies           |  |
|--------------------------|  |
| Do you need extra BI-FB kit supplies? | Yes | No |
| * must provide value     |  |

| Comments                  |  |
|---------------------------|  |

[Submit]
- 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**

<table>
<thead>
<tr>
<th>Specimen Collection Kit</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BI-FB Collection Kit</td>
<td></td>
</tr>
<tr>
<td>BI-FB LP Kit</td>
<td></td>
</tr>
<tr>
<td>BI-FB CSF Kit</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Shipping Kits</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NCRAD Ambient Shipping Kit</td>
<td></td>
</tr>
<tr>
<td>NCRAD Frozen Shipping Kit (holds 8 subjects)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Supplemental Kits</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BI-FB Supplemental Supply Kit</td>
<td></td>
</tr>
<tr>
<td>BI-FB CSF Supplemental Supply Kit</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extra Supplies</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you need extra BI-FB kit supplies?</td>
<td>Yes</td>
</tr>
<tr>
<td>Comments</td>
<td></td>
</tr>
</tbody>
</table>

Each BI-FB Collection Kit Contains:
- 2 Sodium Heparin (green-top) blood collection tube (10 ml)
- 2 EDTA (purple-top) blood collection tube (10 ml)
- 1 Serum (red-top) blood collection tube (10 ml)
- 1 PAXgene blood collection tube (2.5 ml)
- 15 ml conical polypropylene tube individually wrapped
- 6 Cryovial tube with purple cap (2.0 ml)
- 3 Cryovial tube with red cap (2.0 ml)
- 2 Cryovial tube with blue cap (2.0 ml)
- 2 Cryovial tube with clear cap (2.0 ml)
- 13 Pre-printed Specimen Label
- 7 Pre-printed Kit Number Label
- 1 Label for handwritten Patient ID
- 1 Cryovial box (holds up to 25 cryovials)
- 1 Bubble wrap tube sleeve for PAXgene™ tube
Specimen Labels
Three Label Types

- **Kit Number**
  - 300001

- **Patient ID**

- **Specimen**
  - 0001234567
  - BIFB
  - PBMC
  - Kit #: 300001
Kit Number Labels

- Used to track patient samples and provide quality assurance – Will be placed on the following locations:
  1. Blood and CSF Sample and Shipment Notification Forms
  2. Cryoboxes that house aliquots during shipping
  3. One extra label provided

Provided by NCRAD in the kits
Patient ID Label

- Subjects will be identified by their PT ID
- 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
All collection tubes will have two labels:

- The handwritten Patient ID label
- The specimen label
Collection Tube - BLOOD
Specimen Labels – Serum, Plasma, and Buffy Coat

Only one label to be placed on ALL cryovials

- **Serum**
  - From Serum tube
- **Plasma**
  - From EDTA tube
- **Buffy Coat**
  - From EDTA tube
Specimen Labels – Serum, Plasma, Buffy Coat, and CSF

- Specimen tube label only
- Please place barcode near cap
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
Specimen Labels - CSF

- CSF label
  - CSF aliquots
- Separate Kit Number for CSF collection
  - Kit number will differ from kit number used for the blood samples for the same subject at the same visit

0001111123
BIFB
CSF
Kit #: 300002
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>![Tube Image]</td>
</tr>
<tr>
<td>2. EDTA (Purple-Top) Tube (10 ml)</td>
<td>X2</td>
<td>![Tube Image]</td>
</tr>
<tr>
<td>3. Serum (Red-Top) Tube (10 ml)</td>
<td>x1</td>
<td>![Tube Image]</td>
</tr>
<tr>
<td>4. PAXgene™ Tube (2.5 ml)</td>
<td>x1</td>
<td>![Tube 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>![Purple Cap Image]</td>
</tr>
<tr>
<td>Clear</td>
<td>Buffy Coat</td>
<td>![Clear Buffy Coat Image]</td>
</tr>
<tr>
<td>Red</td>
<td>Serum</td>
<td>![Red Cap Image]</td>
</tr>
<tr>
<td>Clear</td>
<td>CSF</td>
<td>![Clear 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: Store tubes at room temp.
Step Two: Collect blood in Sodium Heparin 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 sample.
Step Four: Store tubes at room temp. until shipment.

Label tubes with pre-printed Patient ID and specimen labels prior to blood draw.
Ship ambient same day as blood draw.
Plasma Collection

Create up to 7 aliquots (purple caps + blue cap)

Please see the NCRAD YouTube Channel for tutorials:
http://kits.iu.edu/artfl-lefftds/videos
Buffy Coat Collection

NCRAD Tutorials: http://kits.iu.edu/artfl-lefftds/videos
Plasma/Buffy Coat Collection and Processing

**Step One**
- Store tubes at room temp.
- Each tube should be labeled with Patient ID and Kit Number Labels.

**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 tube 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.
- 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. Repeat for second tube.
- Store plasma aliquots upright at -80°C until shipment to NCRAD.

**Step Seven**
- Label clear-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.

**Step Eight**
- Spin, aliquot, and freeze all plasma and buffy coat aliquots within 2 hours of collection.
Serum Tube

(Immediately after blood draw – pictured below)

** 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.
- Each tube should be labeled with ID Label and Specimen Label.

**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.
- Within 60 minutes of blood draw, centrifuge samples at 2000 x g for 10 minutes at 4°C.

**Step Five**
- Label three red-capped cryovials and one blue-capped cryovial with “SERUM” labels.
- Aliquot 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 2 hours of collection.
RNA PAXgene™ Tubes for RNA

Please see the NCRAD YouTube Channel for tutorials:
http://kits.iu.edu/artfl-lefftds/videos
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.
CSF Collection and Processing
CSF Collection and Processing

Step One
- Label cryovials with pre-printed specimen labels prior to collection.

Step Two
- Pre-chill all cryovials on wet ice.

Step Three
- Collect initial 1-2 ml CSF for local lab testing.
  - If bloody, collect CSF until cleared of blood.
  - If not bloody, transfer into the yellow-capped cryovial. Send to local lab for cell count.

Step Four
- Collect 15-20 ml total (including the 1-2 ml for local lab testing) into two 15 ml conical tubes.
- Place samples upright on wet ice until centrifugation begins.

Step Five
- Within 15 mins of collection, centrifuge samples at 4°C at 2000 x g for 10 minutes.

Step Six
- Using a clean transfer pipette, transfer all CSF into a new 50 ml conical tube leaving the pellet in the bottom.
- Gently invert the 50 ml conical tube 3-4 times to mix the sample.
- Aliquot 1.5 ml into the clear-capped cryovials.
- If residual aliquot is created, place sample into the blue-capped cryovial and document the specimen number and volume on Sample Form.
- Store CSF aliquots upright at -80°C until shipment.
- Spin, aliquot, and freeze aliquots within 2 hours of collection.
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>Baseline Visit</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>Baseline Visit</td>
<td>Plasma</td>
<td>1.5 ml plasma aliquots</td>
<td>Up to 7</td>
<td>Frozen</td>
</tr>
<tr>
<td></td>
<td>Baseline Visit</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>Baseline Visit</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>Baseline Visit</td>
<td>Whole Blood</td>
<td>N/A</td>
<td>N/A</td>
<td>Frozen</td>
</tr>
<tr>
<td>Sterile Container</td>
<td>Baseline Visit</td>
<td>CSF</td>
<td>1.5 ml CSF aliquots</td>
<td>Up to 14</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
Frozen Shipment Packaging

- Use the biohazard bag to package the 25-Slot cryobox and PAXgene™ tube (in the 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**

Dry Ice

For Diagnostic or Medical Purposes Only
No Shipping Papers Required

Contains **20** kg of Dry Ice
Blood and/or CSF 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
# Appendix B: Blood Sample and Shipment Notification Form

**To:** Kelley Faber  
**Email:** alzstudy@iu.edu  
**Fax:** 317-321-2003  
**Phone:** 1-800-556-2839

**From:**  
**Email:**  
**Phone:** 

**Sample Collection:**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sample Type</th>
<th>Volume</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Ship Information:**

<table>
<thead>
<tr>
<th>Tracking</th>
<th>Date Sent</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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# Appendix C: CSF Sample and Shipment Notification Form

**To:** Kelley Faber  
**Email:** alzstudy@iu.edu  
**Fax:** 317-321-2003  
**Phone:** 1-800-556-2839

**From:**  
**Email:**  
**Phone:** 

**Sample Collection:**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sample Type</th>
<th>Volume</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Ship Information:**

<table>
<thead>
<tr>
<th>Tracking</th>
<th>Date Sent</th>
<th>Comments</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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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>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>
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/CSF Sample and Shipment Notification Forms
  - Last time subject ate often left blank/unknown
  Recommendation: Complete Sample Notification forms during the participant study visit as samples are processed.

• Incorrect data reported on Sample and Shipment Notification Forms

• Multiple low volume aliquots
  Recommendation: Lay out cryovials in a row and aliquot in order until plasma/CSF is depleted
Nonconformance Cont.

- All frozen samples for a participant not sent within one shipment box (Serum, plasma, buffy coat, and CSF aliquots should be kept together)
- Aliquots arriving to NCRAD without labels
- Sample forms not faxed or scanned to NCRAD the day before shipment

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

- 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

• Questions?
  • 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