

Biospecimen Exchange for Neurological Disorders

#### Concussion Assessment, Research, and Education for Kids CARE4Kids

BIOSPECIMEN COLLECTION & PROCESSING

## Overview

- 1. Specimen uniformity and quality
- 2. Site Equipment
- 3. Procedures
  - Kit Contents and Ordering
  - Sample Labelling
  - Sample Collection and Processing
  - Shipping Samples
  - Non-Conformance
- 4. Contact Information

# Specimen Uniformity and Quality

GENERAL REMINDERS

## Specimen Standardization and Quality

Most biomarkers are sensitive to *time* and *temperature* 

- Standardization of processing across sites is key
- Specimens must be processed within 2 hours of collection
- Reference the *BioSEND Biomarker Specimen Collection, Processing, and Shipment Manual* as needed
- Do not replace or supplement any kit components without first receiving approval from BioSEND/NINDS

Questions? Email <a href="mailto:biosend@iu.edu">biosend@iu.edu</a>

## Site Consumables and Equipment

Sites will need to supply the following items:

- Gloves
- Alcohol wipes
- Butterfly needles
- Tourniquet
- Gauze pads
- Bandages
- Microcentrifuge tube rack
- Sharps bin and lid

- Crushed ice
- Pipettes and pipette tips
- Centrifuge capable of maintaining 4°C
- -80°C Freezer
- Dry ice

## Procedures

MAINTAINING SPECIMEN UNIFORMITY AND QUALITY

## **Biospecimen Collection Protocol**

| Sample type | Visit  | Tube Provided                          | Blood<br>Volume<br>Collected | Sample<br>Volume      | Samples<br>shipped to<br>BioSEND | Cryovial<br>Color   |
|-------------|--------|--|------------------------------|-----------------------|----------------------------------|---------------------|
| Plasma      | T1, T3 | EDTA tube (purple- top)                | 12ml                         | 500ul per<br>cryovial | Up to 12<br>cryovials            | Purple Cap          |
| Buffy Coat  | T1, T3 |  |                              | 750ul                 | 1 cryovial                       | Clear Cap           |
| Plasma      | T1, T3 | P100                                   | 2ml                          | 200ul per<br>cryovial | Up to 5<br>cryovials             | Lavender<br>Sticker |
| Plasma      | T1, T3 | Tasso™ HemoLink w/ EDTA<br>microtainer | 500ul                        | 200ul per<br>cryovial | 1 cryovial                       | White<br>Sticker    |
| RNA         | Т1, Т3 | PAXgene™ tube                          | 2.5ml                        | N/A                   | 1 tube                           | n/a                 |
| RNA         | T1, T3 | Saliva collection kit                  | N/A                          | N/A                   | N/A                              | N/A                 |

## Kit Contents and Ordering

- All sites will be sent a Supplemental Kit with their first kit shipment
  - Contains extra blood collection tubes and processing supplies
  - May be used to replace items in study visit kits
  - Tasso+ Hemolink<sup>™</sup> devices will be provided with the Supplemental Kit
- Study Visit Kits should be ordered as soon as visits are planned
  - Contains collection, processing, and shipping supplies specific to each visit
  - Includes barcoded labels
  - The supplies/labels in each study visit kit are intended for that visit only

## Kit Contents and Ordering – REDCap Survey

#### http://kits.iu.edu/biosend/care4kids

Order kits online through the Kit Request Module for:

- T1/T3 Blood & Saliva Collection kits
- Saliva kit (without blood supplies)
- Ambient Shipping kit
- Extra Supplies

Please provide as much notice as possible when ordering kits and/or supplies.



#### Kit Contents and Ordering: Confirm Site Info

CARE4Kids Kit Request Module

| Study Site  |  |        |
|---|--|--------|
| * must provide value  | Indiana Univers<br>Select your site                | е<br>- |
| Indiana University School of Medicine   | down list  |        |
| Dept. of Medical & Molecular Genetics<br>351 West 10th Street, TK-318<br>Indianapolis, IN 46202-3002<br>(317) 274-5751<br>cdunifon@iu.edu | Verify contact information<br>and update if needed |        |
| Is the contact name above correct? * must provide value   | <ul> <li>○ Yes</li> <li>● No</li> </ul>            |        |
| New Contact Name * must provide value   | Claire Wegel                                       |        |
| Is the shipping address above correct?<br>* must provide value  | ○ Yes<br>○ No<br>reset                             |        |
| Is the e-mail address above correct?<br>* must provide value  | ○ Yes<br>○ No reset                                |        |
| Is the phone number above correct?<br>* must provide value  | ○ Yes<br>○ No                                      |        |

#### Kit Contents and Ordering: Kit Types

CARE4Kids Kit Request Module

Kits are not specific to a subject or time point. After collection, sites will indicate the subject and time point to which BioSEND should link the samples.

#### Which kits or supplies are you requesting?

This request will be delivered to your site by 06-20-2025.

Please note in Comments if you need this order to arrive sooner. Include date of visit.

\* must provide value

T1/T3 Visit Kit (Blood and Saliva)



Ambient shipping kit (for saliva)

Extra Supplies

Please specify in comments if you need kits before the standard two week shipment time.

How many <u>T1/T3 Visit Kit(s)</u> do you need? \* must provide value

3

## Kit Contents and Ordering: T1/T3 Blood & Saliva Collection kits



## Kit Contents and Ordering: Tasso Hemolink<sup>TM</sup>



### Kit Contents and Ordering: Saliva Supplies



#### Collection Volumes

Total blood volumes

| Sample Type   | Amount |
|---|--------|
| Whole Blood for RNA   | 2.5 ml |
| Whole Blood for Plasma and Buffy Coat   | 12 ml  |
| Whole Blood for Plasma (P100 protease inhibitor tube)                             | 2 ml   |
| Whole Blood for Plasma (Tasso Hemolink <sup>™</sup> device with EDTA microtainer) | 0.5 ml |
| Saliva for RNA  | 1 tube |

## Sample Labelling: Example Labels

#### Labels are provided by Indiana University

- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU *immediately*
- Labelling the tubes during processing prevents sample mix-ups



## Sample Labelling: Label Placement

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



Blood Tube Draw Order



\*Plasma from Tasso™ HemoLink Device collected through non-venipuncture method

#### Sample Collection and Processing: Whole blood RNA



#### Sample Collection and Processing: Plasma & Buffy Coat



#### Sample Collection and Processing: Plasma (P100)





 Using a clean transfer pipet, aliquot 200ul of plasma into each 0.5ml cryotube (lavender dot on top).

X 5

- Generate up to 5 x 200ul plasma aliquots.
- Within 90

   minutes of draw,
   freeze plasma
   and buffy coat
   aliquots <u>upright</u>
   at -80°C. Store at
   -80°C until
   shipment.

#### Tasso+ HemoLink<sup>™</sup> Device

Collection instructions – please follow manufacturer's guide

- Within 30 minutes of blood collection, centrifuge tube for 15 minutes at 1500 RCF (x g) at 4°C.
- Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall.
- Using a disposable tipped micropipette, transfer plasma into the 0.5ml (white dot) cryotube.
- Aliquot 200 ul into cryotube.

#### Saliva for RNA Collection (ship to Penn State)



- Write Care4Kids ID on the Subject ID label and affix it to the saliva tube. **Be careful not to cover preprinted barcode on saliva tube.**
- Place sponge in the back of the mouth between cheek and lower gums where saliva typically pools. Gently rub the gums 10 times in small circles and avoid rubbing the teeth.



- Repeat motion on the opposite side of the mouth between the cheek and lower gums for an additional 10 times.
- 3

**Step Three** 

 Hold the tube upright to prevent the liquid inside the tube from spilling. Unscrew the cap from the collection tube without touching the sponge. BE CAREFUL: cap can stick when performing this step so ensure you have a firm grip before attempting.



 Turn the cap upside down, insert the sponge into the tube and close cap tightly. Invert the capped tube and shake vigorously 15 times.





 Invert the capped tube and shake vigorously 15 times.

#### Sample Collection and Processing: Aliquots

Filling biomarker plasma aliquots:

- Fill as many cryovials as possible to 0.5 ml (Plasma) or 0.2ml (P100 or Tasso)
- Over-filled vials may burst in freezer!
- Ship ALL material to IU, even if final vial is less than standard volume





Sample Collection and Processing: Timeline

Timeline for blood processing



## Sample Collection and Processing: Issue #1

#### **Troubleshooting Blood Collection**

#### Issue #1: Tube with little/no vacuum

- Always check expiration date on the tube before beginning blood draw and discard expired tubes
- Store tubes at "room temperature" extreme temperature can affect vacuum
- Keep extra vacutainer tubes from supplemental kit nearby during blood draw to replace "bad" tubes
- If this is a frequent occurrence, report tube type and lot number to IU.

## Sample Collection and Processing: Issue #2

#### **Troubleshooting Blood Collection**

#### Issue #2: Hemolyzed plasma caused by incorrect collection

| Cause: Blood Collection Methods   | Corrective Action   |  |
|---|---|--|
| Improper venipuncture site  | Draw from median cubital, basalic, and cephalic veins from antecubital region of arm  |  |
| Prolonged tourniquet use  | Tourniquet should be released after no more than 1 min, excessive fist clenching should be avoided  |  |
| Not allowing alcohol to dry on skin before venipuncture                       | Without touching, allow the venipuncture site to air dry  |  |
| Use of too large/small bore needle resulting in excess force applied to blood | Avoid using too small/large needle. Needle size dependent on the subject's physical characteristics & amount of blood to be drawn. Most commonly used sizes are $19 - 23$ . |  |
| Pulling/pushing plunger too fast while drawing/transferring blood             | Avoid drawing the syringe plunger too forcefully when collecting blood  |  |
| Ensure all blood collection assemblies are fitted securely, to avoid frothing |   |  |

For more information, visit: http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk\_Jan2004\_VS7167.pdf

#### Sample Collection and Processing: Issue #2 continued

#### **Troubleshooting Blood Collection**

#### Issue #2: Hemolyzed plasma caused by incorrect processing

| Cause: Sample Processing Methods                | Corrective Actions  |
|---|---|
| Vigorous mixing/shaking                         | Gently invert blood collection tube when mixing additive<br>with specimen, follow guidelines in Biologics Manual<br>regarding number of times to invert each type of tube |
| Not allowing serum to clot for recommended time | Serum tubes without clot activator should be allowed to clot<br>for 60 min in a vertical position   |
| Exposure to excessive heat or cold              | Keep samples at ambient temperature until processing  |
| Prolonged contact of serum/plasma with cells    | Do not store uncentrifuged samples beyond recommended time  |

For more information, visit: http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk\_Jan2004\_VS7167.pdf

Specimen Collection and Processing Form

#### Direct Link:

https://redcap.link/Care4KIDSSampleF orm

First page captures basic subject and visit information

## BioSEND

| C Returning? |  |  |
|--------------|--|--|
| AAA          |  |  |
| <b></b>      |  |  |

Biospecimen Exchange for Neurological Disorders Protocol: Care4Kids

Please complete the Specimen Collection and Processing Form, below.

|   | Page 1 of 2            |
|---|------------------------|
| Study Site  | ►                      |
| Email address of staff member completing this form  |                        |
| Note: A copy of the completed sample form and the shipping<br>manifests will be sent to this address. |                        |
| CARE4Kids Subject ID:   | 8 characters remaining |
| Subject's sex (used for DNA quality control)  | ~                      |
| Visit   | ~                      |
| IU Kit Number   | 7 characters remaining |
| Next Pa   | ige >>                 |
| Save & Retu   | urn Later              |

Second page captures processing information

#### **Blood Collection and Processing**

| Date of venipuncture blood collection      | Today M-D-Y        |
|--|--------------------|
| Time of venipuncture blood collection      | Use 24 Hour clock. |
| Was EMLA used?                             | Yes                |
|  | No                 |
| Date participant last ate                  | Today M-D-Y        |
| Time participant last ate                  | Use 24 Hour clock. |
| 1. RNA (PAXGene™ tube, 2.5 mL)             |                    |
| Was blood collected for RNA?               | Yes                |
|  | No                 |
| Number of PAXGene™ tubes collected for RNA |                    |

PDF form of responses will be emailed to you. <u>Print a copy of the</u> <u>Frozen Shipping Manifest and</u> <u>include with shipment to BioSEND.</u>

#### Unnuentia **CARE4Kids Frozen Shipping Manifest** Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Frozen Shipping Manifest will be emailed to you for Subject [subj id]. Please print a copy of that document and include it in the Kit #[kit num] shipping container. Study Site: O Children's National Hospital Seattle Children's Hospital O University of California - Los Angeles O University of Rochester O University of Texas - Southwestern O Wake Forest University O Washington University CARE4Kids Subject ID: 0 T1 0 T3 Visit: IU Kit Number: Date of blood collection: PLASMA EDTA Number of PLASMA EDTA aliquots shipped: Number of BUFFY COAT aliquots shipped: PLASMA P100 Number of PLASMA P100 aliquots shipped: PLASMA TASSO Number of PLASMA TASSO aliquots shipped:

Page 1

**RNA** 

Number of PAXGene<sup>™</sup> tubes shipped

PDF form of responses will be emailed to you. <u>Print a copy of the</u> <u>Ambient Shipping Manifest and</u> <u>include with shipment to Penn</u> <u>State.</u>

| Confi | dential  |  |               |             |
|-------|--|--|---------------|-------------|
|       | CARE4KIDs Ambient Shipping M   | anifest  |               | Page 1      |
|       | Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Ambient<br>Shipping Manifest will be emailed to you for Subject [subj_id]. |  |               | the Ambient |
|       | Please print a copy of that document and include it in the Kit #[kit_num] shipping container to ship to PENN STATE.  |  |               | PENN STATE. |
|       | Study Site:  |  |               |             |
|       | CARE4KIDS Subject ID:  |  |               |             |
|       | Subject Sex (used for DNA quality control):  |  |               |             |
|       | Visit:   |  |               |             |
|       | IU Kit Number:   |  |               |             |
|       | Date/Time of saliva collection:  |  |               |             |
|       | Date participant last ate  |  |               |             |
|       | Time participant last ate  |  |               |             |
|       | Number of saliva sample collected  |  |               |             |
| 1     | Shipping Information - Please Complete.  |  |               |             |
|       | Saliva samples should be sent to Penn State.   |  |               |             |
| -     | Date of Shipment   |  |               |             |
|       | Did/will you use the IU UPS interface to generate the<br>shipping label?   | O Yes<br>O No                                  |               |             |
|       | Which shipping service did you use?  | O UPS<br>O FedEx<br>O World Courier<br>O Other |               |             |
|       | What is the shipment tracking number?  |  |               |             |
|       |  |  |               |             |
|       | 06/03/2025 1:18pm  | proje  | ectredcap.org | REDCap      |

Submission of the Shipping Manifest portion of the form serves as shipment notification to BioSEND

- <u>Must</u> be completed prior to shipment
- If samples are shipped and not received, BioSEND will followup with courier. It is recommended that sites also track shipment to ensure safe delivery

## Shipping Frozen Samples: Tips

Packing and Shipping Frozen Samples

- All blood samples ship frozen on dry ice
- Frozen samples should be shipped *only* Monday through Wednesday
- Always fill carton to **top** with dry ice
- Do not pack shipment until the day of pickup



#### Shipping Samples

Packing and Shipping Frozen Samples Class 9 Dry Ice Label should not be covered with other stickers and must be completed, or UPS will reject/return your package!



## Shipping Samples: Frozen

Do not ship more than 4 biohazard bags in a single shipper (equivalent to two subject-visits).

- Allows room for dry ice to keep samples frozen in transit
- Minimizes loss in the rare but unfortunate event of courier issues



### Shipping Samples – UPS: <a href="https://kits.iu.edu/UPS">https://kits.iu.edu/UPS</a>



#### Shipping Samples via UPS

IU UPS ShipExec Shipping Portal

- Print out UPS air waybill
- Ensure all elements (barcode, return address, etc.) printed clearly
- Fold and insert UPS air waybill into clear plastic sleeve on package

2 LBS JOHN SMITH 1 OF 1 INDIANA UNIVERSITY 410 WEST 10TH STREET RS INDIANAPOLIS IN 46202 SHIP TO: SCHOOL OF MEDICINE 317-278-2694 INDIANA UNIVERSITY TK 217 351 W 10TH ST **INDIANAPOLIS IN 46202** IN 461 9-01 UPS NEXT DAY AIR TRACKING #: 1Z 976 R8W 84 3985 8595 BILLING: P/P **DESC: Biological Specimens** RETURN SERVICE Reference No.1: 4087277 XOL 20.03.09 NV45 83.0A 12/2019

## Shipping Ambient Samples: Tips

#### Packing and Shipping Ambient Samples

- Saliva samples ship ambient to Penn State with the pre-printed UPS airway bill provided by BioSEND
- Ambient Saliva samples should be batched and shipped to Penn State every 3 weeks
- Please review Appendix R (Saliva Collection for RNA) and Appendix L (Ambient Shipping Instructions).



Shipping Samples via Pre-Printed airwaybill

Ambient Saliva shipping to Penn State

- Please make sure to mark Ambient Shipping Manifest is marked shipped in RedCap and print a copy to include with shipment.
- Place saliva tube(s) in biohazard bag(s) and place in ambient shipper.
- Place the ambient shipper and ambient shipping manifest(s) in the UPS clinpak and seal.
- Attach the UPS pre-printed airway bill addressed to Penn State, UN3373, and Human example label to the clinpak and ship.



## Shipping Samples: Closures

| Date                                 | Holiday                     |
|--------------------------------------|-----------------------------|
| January 1                            | New Year's Day              |
| 3 <sup>rd</sup> Monday in January    | Martin Luther King, Jr Day  |
| 4 <sup>th</sup> Monday in May        | Memorial Day                |
| June 19                              | Juneteenth (observed)       |
| July 4                               | Independence Day (observed) |
| 1 <sup>st</sup> Monday in September  | Labor Day                   |
| 4 <sup>th</sup> Thursday in November | Thanksgiving                |
| 4 <sup>th</sup> Friday in November   | Friday after Thanksgiving   |
| December 25                          | Christmas                   |

Please also consider weather when shipping. UPS will post service updates on their webpage. Reach out to BioSEND if you an unsure if it is safe to ship.

## Non-Conformance

Non-conformance to standard procedures may reduce the utility of the biospecimens:

- Not processing plasma within 2 hours of collection allows for breakdown of certain proteins and small molecules
- Over/under centrifuging changes specimen composition



## Non-Conformance Reporting con't

Most common non-conformance issues:

- Shipment notification not sent
- Samples shipped for weekend/holiday delivery
- Sample form incomplete/inaccurate
- Low volume
- Unlabeled or mislabeled tube(s)
- Sample hemolysis



## Non-Conformance and Inventory Reporting

Most common non-conformance issues:

- BioSEND will notify sites directly of any issues upon receipt
- BioSEND will email sites a monthly inventory report of all samples received from that site to date
- If you are experiencing issues, please reach out to us for help! It is much easier to prevent an issue before sample collection & shipment than trying to fix it after the fact

#### BioSEND.org

On the website, you can:

- Access your study's kit request module and sample submission form
- Download the most recent version of the Manual of Procedures
- View a recording of this training
- Find information about holiday closures
- Access shipping resources

#### Study Resources

#### KIT REQUEST MODULE

Please follow the below link to access the Kit Request Module. This link will direct you to a REDCap database where study coordinators and staff may request kits, individual supplies, and/or labels. Please allow a total of two weeks for kit requests to be fulfilled.

#### Kit Request System →

SPECIMEN COLLECTION AND PROCESSING FORM

Please use the below link to access the collection and processing form for this protocol. This form must be completed prior to shipment. We also ask that all shipments include a physical copy of the shipping manifest portion of the form.

Specimen Collection and Processing Form →

#### MANUAL OF PROCEDURES

The below downloadable manual was created specifically for the DxCTEII study. Please feel free to explore the manual through the hyperlinked 'Table of Contents'. Questions concerning any part of the manual may be directed to **biosend@iu.edu** for further clarification.

#### Manual of Procedures 🛓

#### SAMPLE SHIPPING

BioSEND can receive samples Monday-Friday, excluding holidays. Frozen samples should be shipped M-W. Ambient samples may be shipped on Th.

Generate UPS airbill or schedule pickup  $\rightarrow$ Check holiday closures  $\rightarrow$ What do I do for Friday blood draws  $\rightarrow$ 

#### TRAINING SLIDES

These slides correspond to the BioSEND DxCTEII protocol training. Training is available upon request by contacting biosend@iu.edu.

#### Training Slides 🛃

### Contacts

Indiana University

General Questions/Shipment Notifications:

<u>biosend@iu.edu</u>

317-278-6158

Request kits:

http://kits.iu.edu/biosend/care4kids