



Biospecimen Exchange for Neurological Disorders

An Intermediate Expanded Access Protocol with CNM-Au8  
for Amyotrophic Lateral Sclerosis for Protocol  
CNMAU8.EAPo<sub>4</sub> (CNMAu8.EAPo<sub>4</sub>)

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BIOSPECIMEN COLLECTION & PROCESSING

# Overview

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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

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GENERAL REMINDERS

# Specimen Standardization and Quality

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

- Standardization of processing across sites is key
- Specimens must be processed and frozen within specified windows (one hour for whole blood, two hours for all other sample types)
- 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 [biosend@iu.edu](mailto:biosend@iu.edu)

# Site Consumables and Equipment

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*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 1500 RCF (x g)
- -80°C Freezer (for in-clinic storage)
- Dry ice

# Procedures

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MAINTAINING SPECIMEN UNIFORMITY AND QUALITY

# Biospecimen Collection Protocol

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Samples collected at visit weeks 1 (Screening/Baseline), 12, 24, 48, 72, 96, 120, and 144 or ET					
Draw Order	Collection Tube	Specimen Type	Aliquot Volume	Total Number of Aliquots	Cryovial Cap Color
1	1 PST LiHep (Green-top) Tube (4.5ml)	Plasma for creatinine testing	2ml	1	Orange
2	2 EDTA (purple-top) Tubes (10ml)	Plasma for Quanterix Nfl and UCHL1 analysis	1.5ml	6	Purple
		Buffy Coat for DNA extraction	~750ul	2	Clear
3	1 EDTA (purple-top) Tube (3ml)	Whole Blood for NadMED analysis	0.5ml	6	Green

\*For females of child-bearing potential only: A urine pregnancy test should take place at the Screening/Baseline visit and as clinically indicated thereafter. Pregnancy tests and urine should **not** be sent to BioSEND.

# Kit Contents and Ordering

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- 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
- 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



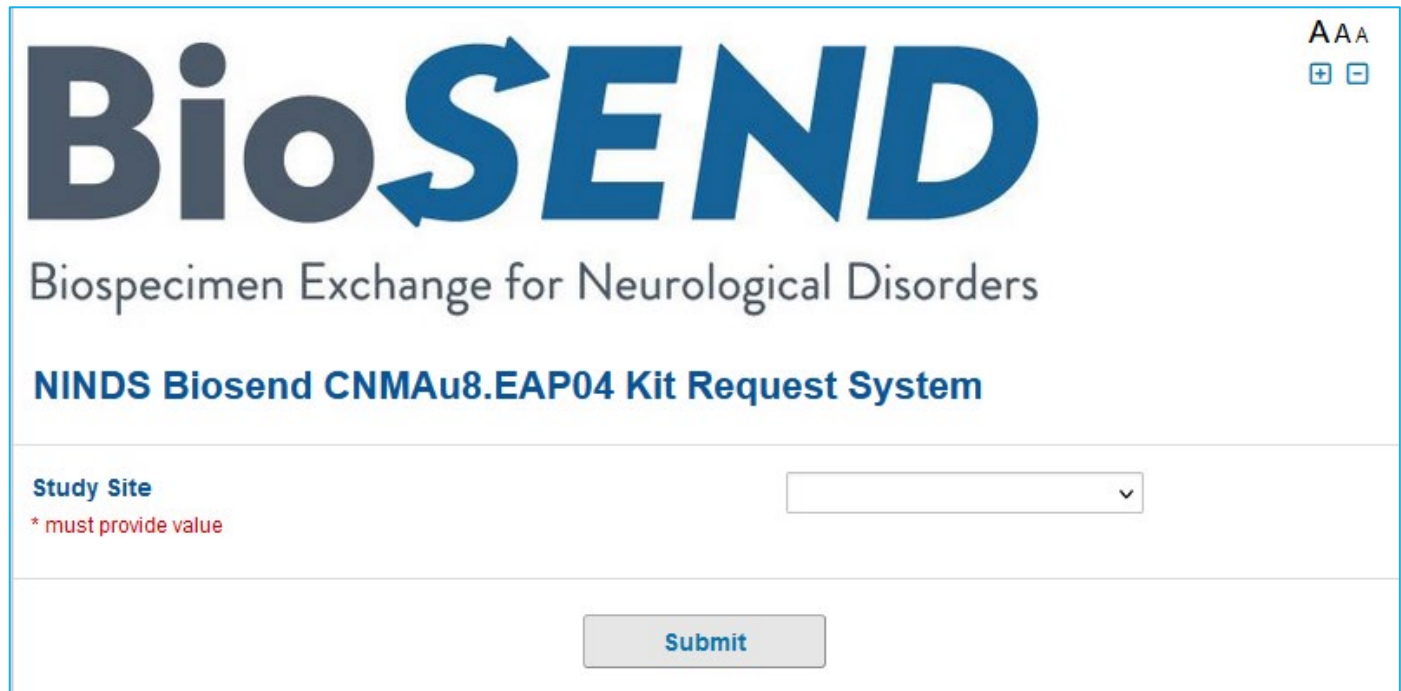
# Kit Contents and Ordering – REDCap Survey

<http://kits.iu.edu/biosend/CNMAu8EAP04>

Order kits online through the Kit Request Module for:

- Blood Kits
- Supplemental Kit
- Extra Supplies

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



The screenshot shows the 'BioSEND' logo with the tagline 'Biospecimen Exchange for Neurological Disorders'. Below this is the title 'NINDS Biosend CNMAu8.EAP04 Kit Request System'. A form field labeled 'Study Site' is present, with a dropdown arrow and a red asterisk indicating it is required. A 'Submit' button is located at the bottom right of the form area.

**BioSEND**  
Biospecimen Exchange for Neurological Disorders

**NINDS Biosend CNMAu8.EAP04 Kit Request System**

Study Site  ▼  
\* must provide value

**Submit**

# Kit Contents and Ordering: Confirm Site Info

Confirm where kits/supplies should be shipped

<b>Study Site</b> <small>* must provide value</small>	Indiana University	Select your site from the drop-down list
Indiana University School of Medicine  Carolyn Dunifon Dept. of Medical & Molecular Genetics 351 West 10th Street, TK-318 Indianapolis, IN 46202-3002 (317) 274-5751 cdunifon@iu.edu		
<b>Is the contact name above correct?</b> <small>* must provide value</small>	<input type="radio"/> Yes <input checked="" type="radio"/> No	Verify contact information and update if needed
<a href="#">reset</a>		
<b>New Contact Name</b> <small>* must provide value</small>	Claire Wegel	
<b>Is the shipping address above correct?</b> <small>* must provide value</small>	<input type="radio"/> Yes <input type="radio"/> No	<a href="#">reset</a>
<b>Is the e-mail address above correct?</b> <small>* must provide value</small>	<input type="radio"/> Yes <input type="radio"/> No	<a href="#">reset</a>
<b>Is the phone number above correct?</b> <small>* must provide value</small>	<input type="radio"/> Yes <input type="radio"/> No	<a href="#">reset</a>

# Kit Contents and Ordering: Kit Types

## 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.

**Kit Type**  
**\*\*Please allow two weeks for shipment\*\***  
\* must provide value

+

Remote Collection & Shipping Kit

+

In Clinic Collection Kit

+

In Clinic Shipping Kit - Standard Size

+

In Clinic Shipping Kit - Bulk Size

+

Extra Supplies

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

# Kit Contents and Ordering: Kit Breakdown

Select a kit type to view contents

**Kit Type**  
**\*\*Please allow two weeks for shipment\*\***  
\* must provide value

+ Remote Collection & Shipping Kit

☒ In Clinic Collection Kit

+ In Clinic Shipping Kit - Standard Size

+ In Clinic Shipping Kit - Bulk Size

+ Extra Supplies

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

**In Clinic Collection Kit Quantity**  
\* must provide value

3

**Comments**

Expand

**Each In Clinic Collection Kit contains:**

Kit contains the supplies to collect samples for one study visit.

Supply	Quantity
Cryovial (Sarstedt®) with green cap, 2ml	6
Cryovial (Sarstedt®) with purple cap, 2ml	6
Cryovial (Sarstedt®) with clear cap, 2ml	2
LiHep (plastic) tube, 4.5ml	1
EDTA (plastic) tube, 10ml	2
EDTA (plastic) tube, 4ml	1
Disposable pipet, 3ml	3
Cryobox, 25 cell	1
Hcg pregnancy dipstick test	1
Urine cup	1
Label set (kit & specimen labels)	1

Kit contents of selected kit will appear at the bottom of the page

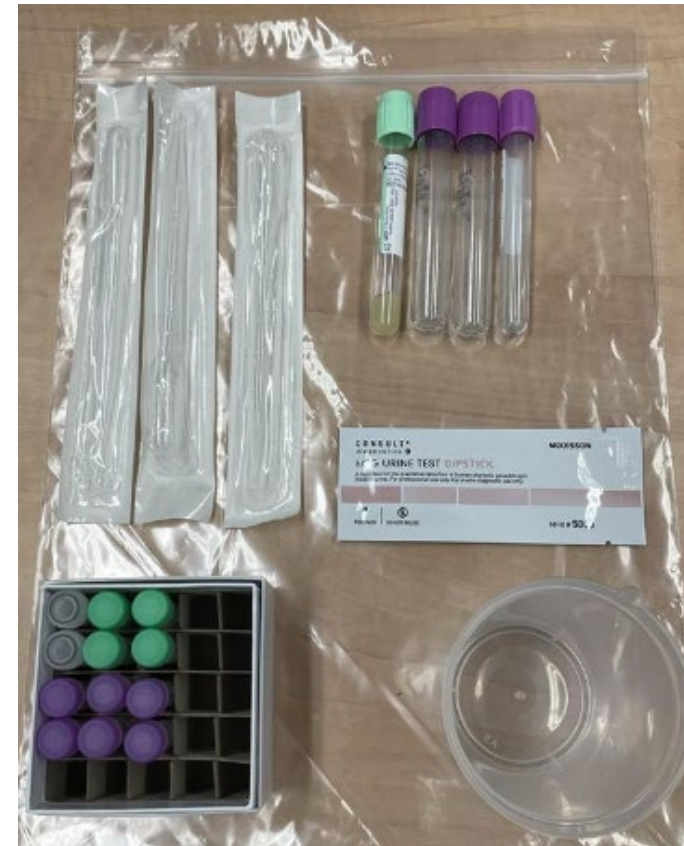
# Kit Contents and Ordering: Remote Collection & Shipping Kit

- These kits will contain the supplies to collect and ship the samples from one participant-visit, as samples will be shipped as they are collected.



# Kit Contents and Ordering: In-Clinic Collection Kit

- Because in-clinic samples may be stored and batch shipped on a quarterly basis, In-Clinic Collection Kits will contain only the supplies to collect and process samples. Shipping kits are ordered separately (see next slide).



# Kit Contents and Ordering: In-Clinic Shipping Kit Options

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## STANDARD SIZE SHIPPING KIT

- This kit contains the supplies to ship the samples from up to two study visits (that is, up to 4 biohazard bags may be shipped in a single shipper).

## BULK SIZE SHIPPING KIT

This kit contains the supplies to ship the samples from up to four study visits (that is, up to 8 biohazard bags may be shipped in a single shipper).

# Kit and Supply Ordering

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- Click “Submit” to send order to BioSEND; staff will confirm receipt of your order
- Please allow two week turn-around time for kit shipments
- If urgent request needed, please note date needed by in comments and email BioSEND. We cannot guarantee urgent orders, but we will do our best to accommodate.
- BioSEND will send confirmation of shipment and tracking number when supplies ship

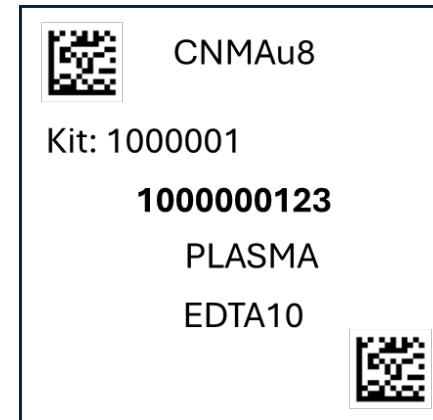


# Sample Labeling: Example Labels

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## 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*
- Note that you will have extra labels. If you do not need the extra labels, please dispose of them.
- Labelling the tubes during processing prevents sample mix-ups

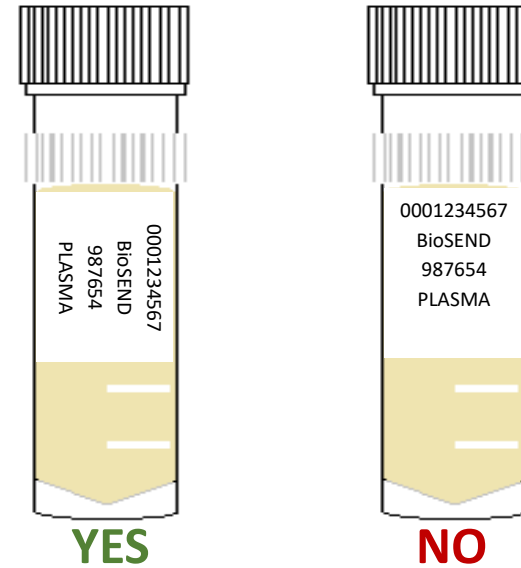


# Sample Labeling: Label Placement

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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



# Sample Collection and Processing

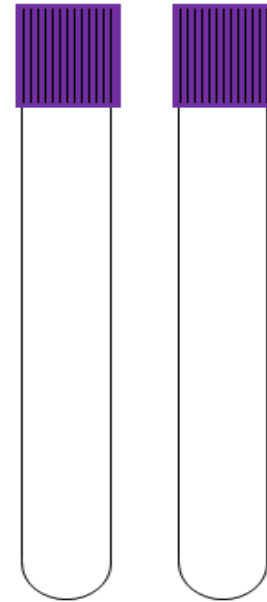
Blood Tube Draw Order

1 x 4.5ml PST  
LiHep for  
creatinine testing



**1**

2 x 10ml EDTA  
for plasma and  
buffy coat



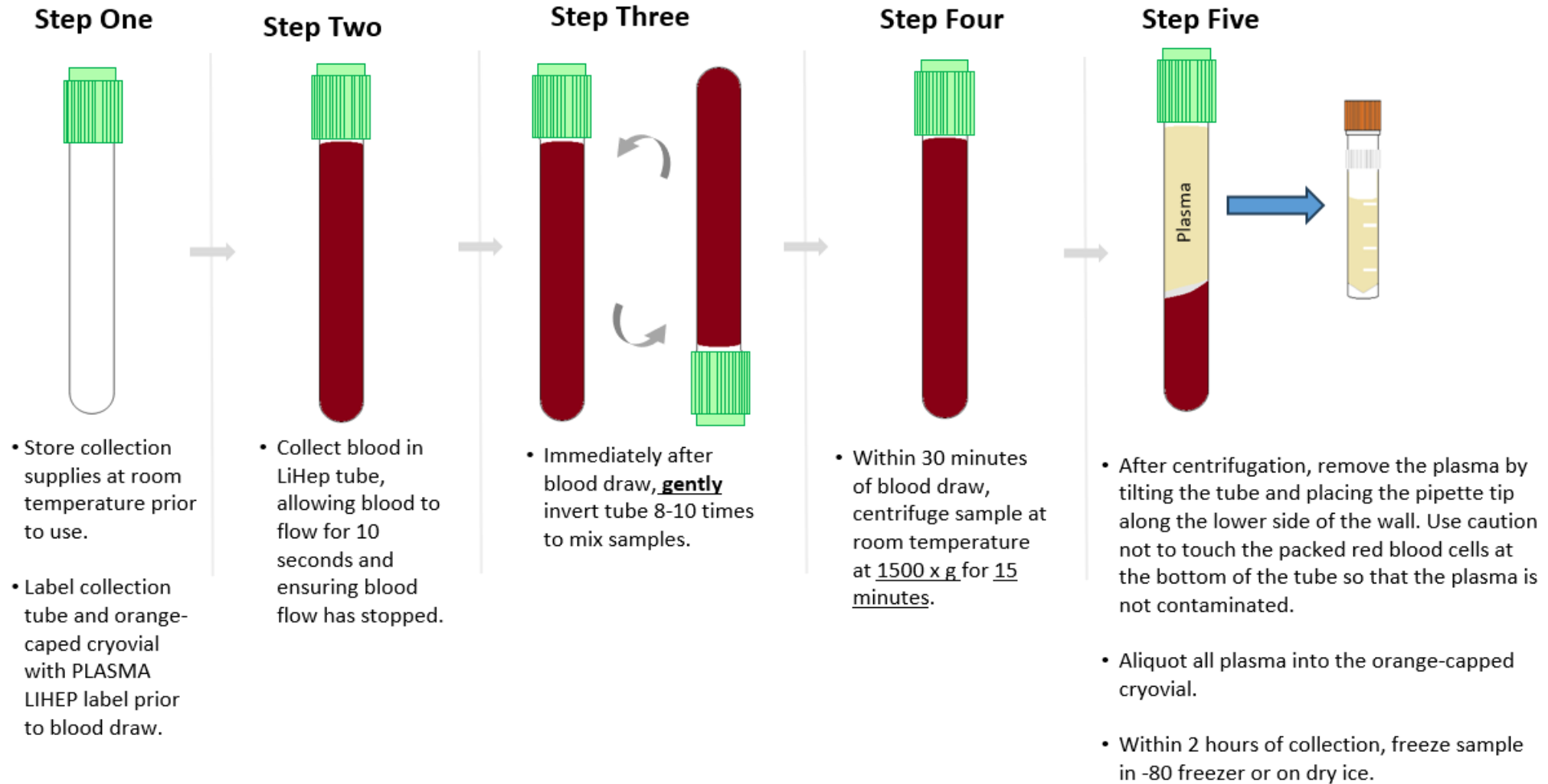
**2**

1 x 3ml EDTA  
for whole  
blood

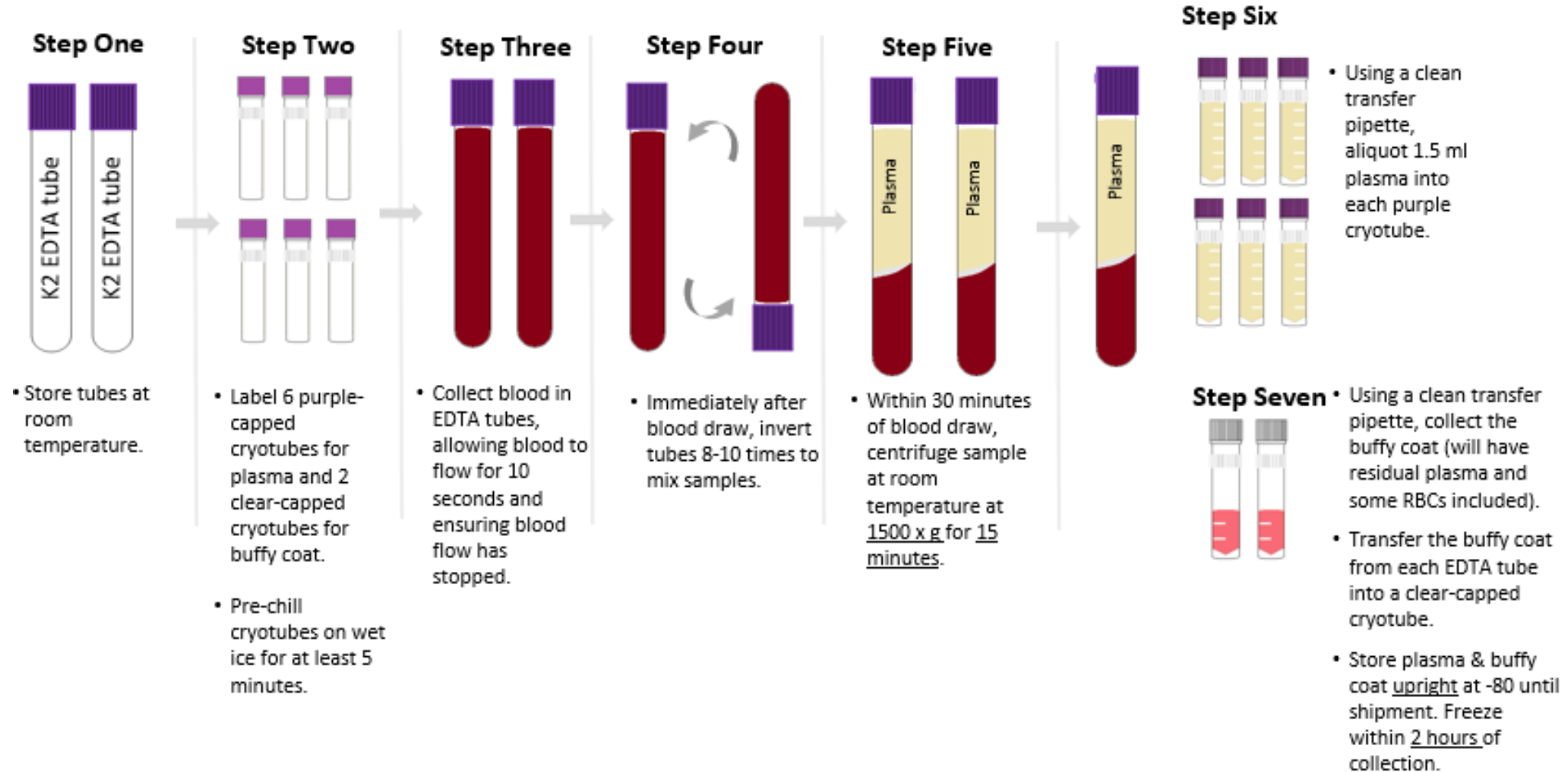


**3**

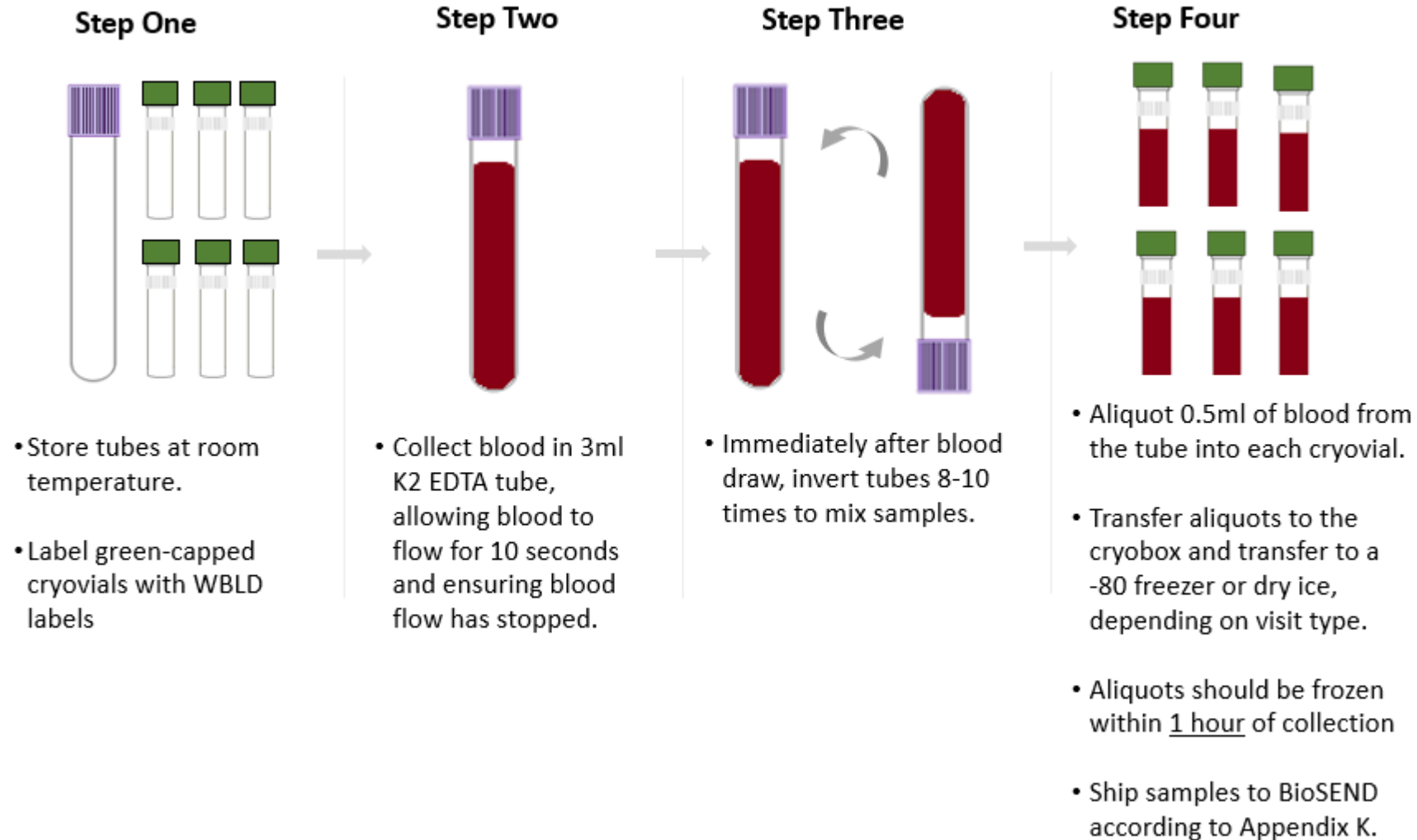
# Sample Collection and Processing: 4.5ml PST LiHep



# Sample Collection and Processing: Plasma & Buffy Coat



# Sample Collection and Processing: Whole Blood

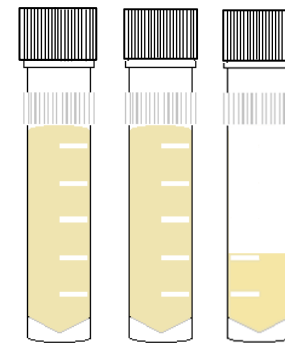


# Sample Collection and Processing: Aliquots

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Filling biomarker plasma and whole blood aliquots:

- In the event of a low volume draw, please generate as many standard-sized aliquots as possible. Fewer standard sized aliquots (with potentially one residual aliquot) is preferable to more aliquots with non-standard volume.
- Over-filled vials may burst in freezer!
- Ship ALL material to IU, even if final vial is less than standard volume



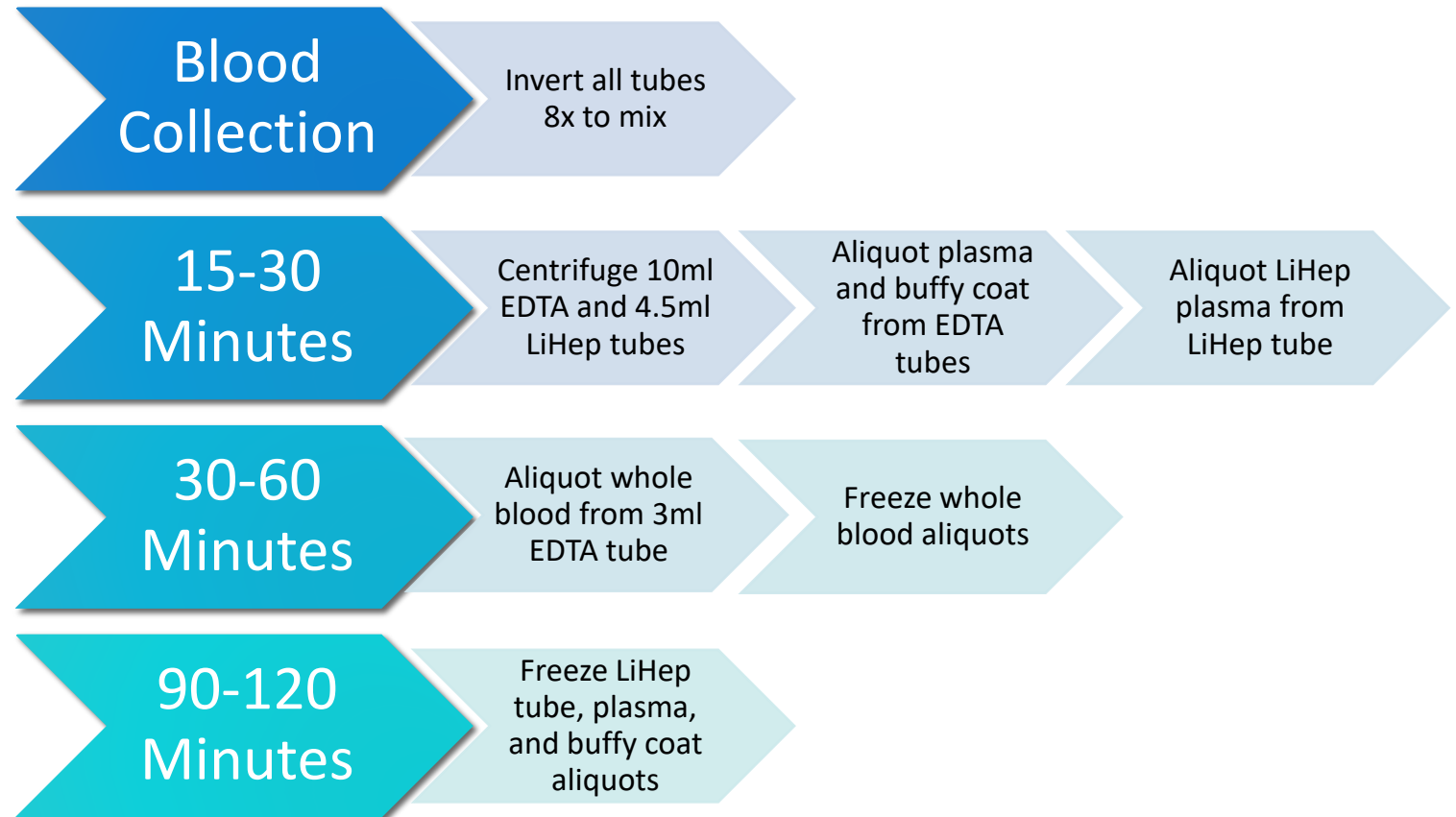
YES



NO

# Sample Collection and Processing: Timeline

Timeline for blood processing





# Sample Collection and Processing: Issue #1

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## 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

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## 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](http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf)

# Sample Collection and Processing: Issue #2 continued

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## 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 with specimen, follow guidelines in Biologics Manual 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 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](http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf)

# Sample Collection and Processing Form

Direct link:

<http://kits.iu.edu/biosend/CNMAu8EAP04SampleForm>

First page captures basic participant and visit information

Returning?

AAA

BioSEND

Biospecimen Exchange for Neurological Disorders

Please complete the Specimen Collection and Processing Form, below.

Page 1 of 2

Intermediate Expanded Access Protocol CNMAu8.EAP04

Study Site

Email address of staff member completing this form

*Note: A copy of the completed sample form and the shipping manifest will be sent to this address.*

Participant ID

Sex (used for DNA quality control)

Weight

In pounds

Height

In inches

Visit

Kit Number

6-digit number on specimen labels

# Sample Collection and Processing Form

Direct link:

<http://kits.iu.edu/biosend/CNMAu8EAP04SampleForm>


Second page captures processing information. Protocol instructions, such as centrifugation rate, are pre-populated.

## Blood Collection and Processing

Date of venipuncture blood collection

 Today M-D-Y

Time of venipuncture blood collection

 Now H:M  
Use 24 Hour clock

Date participant last ate

 Today M-D-Y

Time participant last ate

 Now H:M

1. PLASMA for Creatinine Testing (one green-top LiHep tube, 4.5 mL)


Was blood collected and processed for PLASMA LIHEP?

Yes

No

reset

Time of LiHep tube centrifugation

 Now H:M  
Use 24 Hour clock

Duration of LiHep tube centrifugation

15  
minutes

Rate of LiHep tube centrifugation

1500  
x g

Was LiHep tube centrifuged at room temperature?

Yes

No

reset

Time LiHep was placed in freezer

 Now H:M  
24 Hr clock

# Sample Collection and Processing Form

Direct link:

<http://kits.iu.edu/biosend/CNMAu8EAP04SampleForm>

PDF form of responses will be emailed to you. **Print a copy of the Frozen Shipping Manifest and the creatinine req form and include with shipment.**

## CNMAu8 Frozen Shipping Manifest

Page 1

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 123.

Please print a copy of that document and include it in the shipping container.

Response was added on 05/02/2024 11:37am.

Study Site	<input checked="" type="radio"/> Duke University
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Participant ID	123
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Visit:	<input checked="" type="radio"/> 12 Weeks
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Kit Number:	123456
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Date of blood collection:	2024-05-02
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### LiHep TUBE

Number of LiHep tubes shipped:	1
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### PLASMA EDTA

Number of PLASMA EDTA aliquots shipped:	7
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Number of BUFFY COAT aliquots shipped:	3
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### WHOLE BLOOD EDTA

Number of WHOLE BLOOD aliquots shipped:	5
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# Creatinine Req Form

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Patient Name (BioSEND, Kit Number): <b>BioSEND,</b> <span>a.</span>		DOB: <b>01/01/1900</b>	<input type="checkbox"/> STAT	Date/Time of Collection (24hr clock): <span>b.</span>
<span>c.</span> <input type="checkbox"/> <b>M</b> <input type="checkbox"/> <b>F</b>	HEIGHT (inches)/WEIGHT (lbs) <span>d.</span>		Physician: Pathnet, Client	

- a) Kit Number
- b) Date/Time of Collection (24hr clock)
- c) Indicate Male or Female
- d) Height (inches)/Weight (lbs)
- e) Do not enter anything in the Physician, STAT, or DOB fields

# Sample Collection and Processing Form

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

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



# Shipping Frozen Samples: Tips

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## Packing and Shipping Frozen Samples

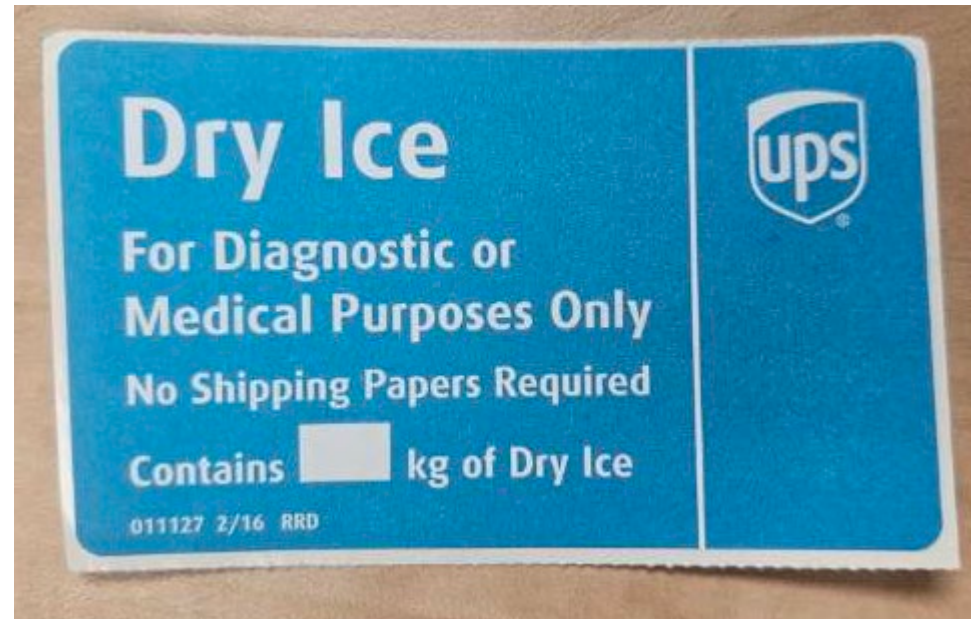
- All samples ship frozen on dry ice
- Frozen samples should be shipped **only** Monday through Wednesday
- Always fill carton to **top** with dry ice
- Do not pack shipment until the day of pickup



# Shipping Samples

Packing and Shipping Frozen  
Samples

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



# Shipping Samples: Frozen

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## Packing and Shipping Frozen Samples



Shipping Samples – UPS: <https://kits.iu.edu/UPS>

The screenshot shows the 'Ship From' and 'Shipment Information' sections of the IU School of Medicine shipping form. Annotations with blue arrows point to specific fields and buttons:

- Search for address:** Points to the search icon in the 'Ship From' section.
- Choose Study:** Points to the 'Study Group' dropdown menu in the 'Shipment Information' section.
- Enter weight:** Points to the 'Weight' and 'Dry Ice Weight' input fields in the 'Shipment Information' section.
- Schedule Pickup:** Points to the 'Pickup Request' button in the 'Shipment Information' section.
- Click "Ship":** Points to the 'Ship' button at the bottom right of the form.

The form contains the following data:

Ship From		Shipment Information	
Code		Study Group	SSBC
Company	Icahn School of Medicine - Mt. Sinai	Weight	20 LB
Contact	Kenny Persaud	Dry Ice Weight	10 LB
Address 1	1425 Madison (Icahn Building)	Description of Return	Biological Specimens
Address 2	Icahn L4 39		
Address 3			
City	New York		
State/Province	NY		
Postal Code	10029		
Country/Territory	United States		

# 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

JOHN SMITH INDIANA UNIVERSITY 410 WEST 10TH STREET INDIANAPOLIS IN 46202	2 LBS	1 OF 1
RS		
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		1
TRACKING #: 1Z 976 R8W 84 3985 8595		
 SAMPLE		
BILLING: P/P DESC: Biological Specimens RETURN SERVICE		
Reference No.1: 4087277		
XOL 20.03.09 NV45 83.0A 12/2019		 TM

# 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 are unsure if it is safe to ship.

# Non-Conformance

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

- Processing samples outside of processing window allows for breakdown of certain proteins and small molecules. Whole blood aliquots should be frozen within 1 hour of collection, while plasma/buffy coat/LiHep should be processed and frozen within 2 hours of collection.
- Over/under centrifuging changes plasma composition



# Non-Conformance Reporting con't

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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

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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](mailto:biosend@iu.edu) for further clarification.

[Manual of Procedures ↓](#)

### TRAINING SLIDES

These slides correspond to the BioSEND DxCTEII protocol training. Training is available upon request by contacting [biosend@iu.edu](mailto:biosend@iu.edu).

[Training Slides ↓](#)

### 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 →](#)  
[Check holiday closures →](#)  
[What do I do for Friday blood draws →](#)

# Contacts

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## Indiana University

General Questions/Shipment Notifications:

[biosend@iu.edu](mailto:biosend@iu.edu)

317-278-6158

Request kits:

<http://kits.iu.edu/biosend/CNMAu8EAP04>